

(12)

(21) 2 456 477

(22) 21.08.2002

(51) Int. Cl. ⁷: A61K 38/00

(85) 04.02.2004

(86) PCT/US02/026525

(87) WO03/015809

(30) 60/314,232 US 21.08.2001
10/225,087 US 20.08.2002

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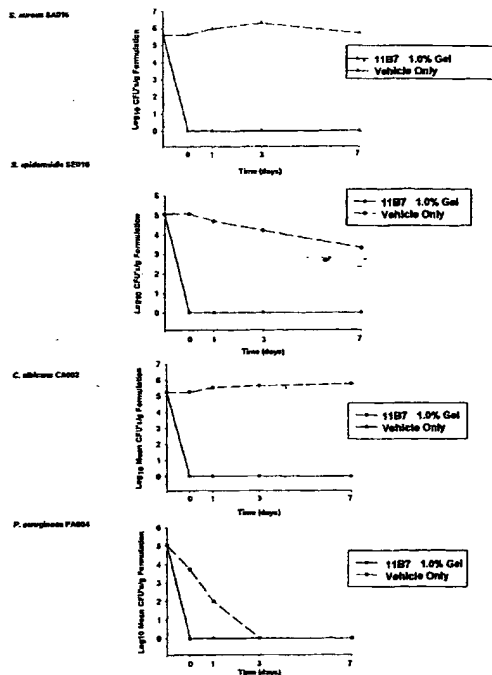
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(54) PEPTIDES CATIONIQUES ANTIMICROBIENS ET FORMULATIONS ASSOCIEES

(54) ANTIMICROBIAL CATIONIC PEPTIDES AND FORMULATIONS THEREOF

(57)

Compositions and methods for making and using therapeutic formulations of antimicrobial cationic peptides are provided. The antimicrobial cationic peptide formulations may be used, for example, in the treatment of microorganism-caused infections, which infections may be systemic, such as a septicemia, or may be localized, such as in acne or an implanted or indwelling medical device.





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CA 2456477 A1 2003/02/27

(21) **2 456 477**

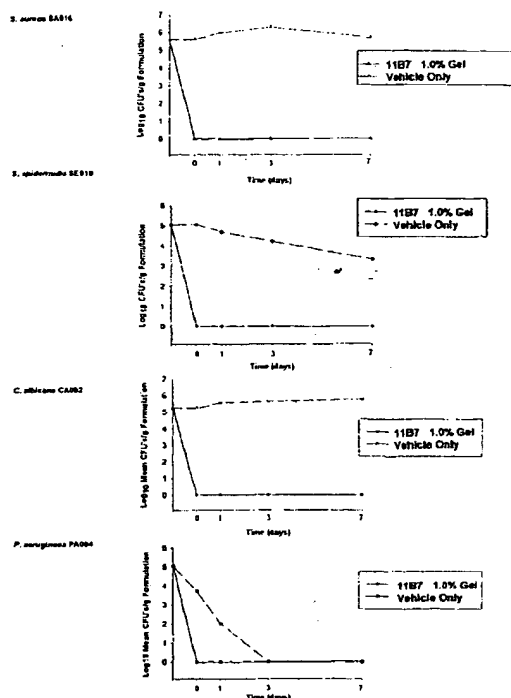
(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2002/08/21
(87) Date publication PCT/PCT Publication Date: 2003/02/27
(85) Entrée phase nationale/National Entry: 2004/02/04
(86) N° demande PCT/PCT Application No.: US 2002/026525
(87) N° publication PCT/PCT Publication No.: 2003/015809
(30) Priorités/Priorities: 2001/08/21 (60/314,232) US;
2002/08/20 (10/225,087) US

(51) Cl.Int.⁷/Int.Cl.⁷ A61K 38/00
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(54) Titre : PEPTIDES CATIONIQUES ANTIMICROBIENS ET FORMULATIONS ASSOCIEES
(54) Title: ANTIMICROBIAL CATIONIC PEPTIDES AND FORMULATIONS THEREOF



(57) Abrégé/Abstract:

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Canada

<http://opic.gc.ca> • Ottawa-Hull K1A 0C9 • <http://cipo.gc.ca>

OPIC • CIPQ 191

OPIC



CIPQ

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 February 2003 (27.02.2003)

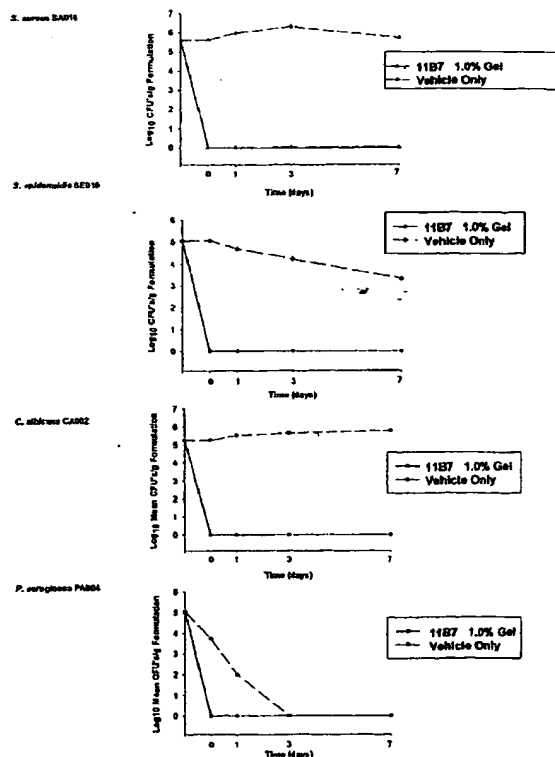
PCT

(10) International Publication Number
WO 03/015809 A2

- (51) International Patent Classification⁷: **A61K 38/00**
- (21) International Application Number: **PCT/US02/26525**
- (22) International Filing Date: **21 August 2002 (21.08.2002)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
60/314,232 21 August 2001 (21.08.2001) US
10/225,087 20 August 2002 (20.08.2002) US
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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,

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(54) Title: **ANTIMICROBIAL CATIONIC PEPTIDES AND FORMULATIONS THEREOF**



(57) Abstract: Compositions and methods for making and using therapeutic formulations of antimicrobial cationic peptides are provided. The antimicrobial cationic peptide formulations may be used, for example, in the treatment of microorganism-caused infections, which infections may be systemic, such as a septicemia, or may be localized, such as in acne or an implanted or indwelling medical device.

WO 03/015809 A2

WO 03/015809 A2

SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VC, VN, YU, ZA, ZM, ZW.

Published:

— without international search report and to be republished
upon receipt of that report

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

ANTIMICROBIAL CATIONIC PEPTIDES AND FORMULATIONS THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to the treatment of
5 infectious disease, and more specifically, to compositions comprising
antimicrobial cationic peptides formulated for therapeutic use.

Description of the Related Art

During this century, modern society has been successful in
controlling infectious disease by, for example, using vaccines, using drugs
10 (such as antibiotics), and using strict public health measures. These advances
have been paralleled by successfully identifying the causative agents of
infectious disease, which agents include bacteria, fungi, protozoa, and viruses.
Thus, aside from a healthy host immune response, antibiotic therapeutic
regimens now represent the primary course of treatment for most infectious
15 diseases in developed countries. In contrast, infectious diseases remain a
serious concern for developing countries, due to the lack of adequate sanitation
and consequent poor hygiene, and for immunocompromised individuals.
However, due to the widespread use of antibiotics, drug-resistance to one or
more antibiotics is becoming an increasingly common problem all over the
20 world for controlling a number of previously treatable infectious diseases (e.g.,
Staphylococcal infections). Accordingly, treatment of nosocomial infections
(i.e., those arising in hospitals) and infections related to indwelling medical
devices is becoming more difficult because of the intractable nature of
infections due to drug-resistant microorganisms, which is a serious and world-
25 wide clinical concern.

A variety of artificial devices to assist in the performance of
various physiological functions have been developed to be inserted into the
human body for short periods, such as catheters, or to be inserted permanently,
such as artificial heart valves; however, the interface between the device and
30 body creates new biological conditions that increase the propensity of infection.
For example, catheter-associated infections may have multiple potential
sources of contaminants, including contaminants in the infusate that is directly
injected, contaminants of the catheter hub where the administration set

attaches to the catheter, contaminants carried hematogenously from remote sources of local infection to colonize the catheter, or contaminants of cutaneous origin that invade the percutaneous tract extralumenally at the time the catheter is inserted or in the days following insertion. Available evidence indicates that the majority of catheter-related bacteremias originate from the cutaneous microflora of the insertion site. Given the evidence for the importance of cutaneous microorganisms in the pathogenesis of intravascular device-related infections, measures to reduce colonization of the insertion site are of great importance in the health care industry.

Another clinical indication of importance is nosocomial infections and, in particular, nosocomial pneumonia and nosocomial sinusitis. Contaminated secretions may be aspirated daily in the tracheobronchial tree, which may lead to pneumonia. Additionally, the risk of nosocomial pneumonia is increased after a tracheostomy is performed and during prolonged endotracheal intubation. Sinusitis has been found to be associated with an increased risk of nosocomial pneumonia, presumably due aspiration of contaminated sinovial fluids into the distal airways. Sinusitis typically arises in the hospital setting among mechanically ventilated patients. Recommended treatments for sinusitis of intubated patients, include removal of the tubes or systemic antibiotics. However, once again, the increase in antibiotic-resistant organisms makes the latter treatment, whether preventative or curative, less efficacious.

Yet another clinical indication, although not life-threatening, is the most common skin disease of adolescence and early adulthood, acne vulgaris, or acne as it is generally called. In addition to psychological effects, such as anxiety, depression and withdrawal from society, studies have also shown that acne vulgaris can directly and significantly affects a patient's quality of life. Antibiotic agents have been extensively used for the treatment of acne for several decades; however, there is a growing concern that with the use of antibiotics to treat acne, drug-resistant microorganisms will inevitably emerge.

To address the issue of ever increasing drug-resistant microorganisms, investigations have turned to new classes of antibiotics, such as antimicrobial peptides. Antimicrobial peptides are found in evolutionarily diverse species including, for example, prokaryotes, plants, insects, and mammals. Antimicrobial peptides may be anionic, but most known antimicrobial peptides are cationic. Multiple families of antimicrobial cationic

peptides are known and these peptides encompass a wide variety of structural motifs, yet all of these cationic peptides have similar physicochemical properties. For example, most known antimicrobial cationic peptides are cationic at neutral pH, are generally less than 10 kDa, and are amphipathically "sided" in solution such that hydrophobic side chains are regionalized. Many antimicrobial cationic peptides are known, including, for example, defensins, cecropins, melittins, magainins, indolicidins, and protegrins. The advantages of cationic peptides are their ability to kill target cells rapidly, their broad spectrum of activity, and their activity against some of the more serious antibiotic-resistant and clinically relevant pathogens. Most importantly, antimicrobial peptide-resistant microorganisms are relatively difficult to select *in vitro*. However, some antimicrobial peptides have been found to be toxic (e.g., bee venom, wasp venom, and scorpion toxin), some have been found to have reduced activity *in vivo* (due to factors such as high mono- and divalent cation concentrations, polyanions, serum, apolipoprotein A-1, serpins, and proteases, although many peptides are not affected by these factors), and some have been found to be not as potent as conventional antibiotics.

Hence, a need exists for identifying modified or derivative antimicrobial peptides with improved activity (and in some cases with reduced toxicity), for formulating such peptides and derivatives thereof for optimal therapeutic use, and for developing therapeutically effective clinical regimens for these cationic peptides. Furthermore, there is a need for formulations that are useful in a variety of clinical indications. The present invention meets such needs, and further provides other related advantages.

BRIEF SUMMARY OF THE INVENTION

The present invention provides antimicrobial cationic peptides, in particular indolicidin peptides and analogs or derivatives thereof, and formulations of such peptides for use in a variety of therapeutic settings, such as in treating or preventing, for example, infectious disease associated with foreign bodies, primary infection sites, or secondary infections arising from a primary disease state.

In one aspect, the present invention provides a composition that comprises an antimicrobial cationic peptide, a viscosity-increasing agent, and a solvent. In certain embodiments the solvent is water, glycerin, propylene glycol, isopropanol, ethanol, or methanol. In certain other embodiments, the solvent is

glycerin at a concentration ranging from about 0.1% to about 20% or from about 9% to about 11%. In still other embodiments, the solvent is propylene glycol at a concentration ranging from about 0.1% to about 20% or from about 9% to about 11%. In yet other embodiments, the solvent comprises at least one of
5 water, glycerin, propylene glycol, isopropanol, ethanol, and methanol. In further embodiments, the solvent comprises at least one of water at a concentration up to 99%, glycerin at a concentration up to 20%, propylene glycol at a concentration up to 20%, ethanol at a concentration up to 99%, and methanol at a concentration up to 99%.

10 In certain embodiments, the viscosity-increasing agent is dextran, polyvinylpyrrolidone, hydroxyethyl cellulose, or hydroxypropyl methylcellulose. In another embodiment, the viscosity-increasing agent is hydroxyethyl cellulose at a concentration ranging from about 0.5% to about 5% or from about 1% to about 3%. In another embodiment, the viscosity-increasing agent is
15 hydroxypropyl methylcellulose at a concentration ranging from about 1% to about 3%. In other embodiments, the viscosity-increasing agent is dextran at a concentration ranging from about 0.1% to about 5% or from about 0.5% to about 1%. In certain other embodiments, where the viscosity-increasing agent is hydroxyethyl cellulose, the composition further comprises a second viscosity-
20 increasing agent of dextran, polyvinylpyrrolidone, or hydroxypropyl methylcellulose. In one embodiment, the second viscosity-increasing agent is polyvinylpyrrolidone. In related embodiments, the polyvinylpyrrolidone is at a concentration ranging from about 0.1% to about 5% or from about 0.5% to about 1%. In another embodiment, the second viscosity-increasing agent is
25 hydroxypropyl methylcellulose. In related embodiments, the hydroxypropyl methylcellulose is at a concentration ranging from about 1% to about 3%. In certain other embodiments, where the viscosity-increasing agent is hydroxypropyl methylcellulose, the composition further comprises a second viscosity-increasing agent of dextran, or dextran at concentration ranging from
30 about 0.1% to about 5% or from about 0.5% to about 1%. In still other embodiments, where the viscosity-increasing agent is hydroxypropyl methylcellulose, the composition further comprises a second viscosity-increasing agent of polyvinylpyrrolidone, or polyvinylpyrrolidone at a concentration ranging from about 0.1% to about 5% or from about 0.5% to
35 about 1%. In certain embodiments, the first viscosity-increasing agent comprises hydroxyethyl cellulose at a concentration up to about 3% and second

viscosity-increasing agent comprises hydroxypropyl methylcellulose at a concentration up to about 3%.

In another embodiment, the present invention provides a composition comprising an antimicrobial cationic peptide, a viscosity-increasing agent, and a solvent, which further comprises a buffering agent. In certain
5 embodiments, the buffering agent is at a concentration ranging from about 1mM to about 200mM. In other embodiments, the buffering agent may comprise a monocarboxylate or a dicarboxylate. In further embodiments, the buffering agent is acetate, fumarate, lactate, malonate, succinate, or tartrate. In yet
10 another embodiment, the composition further comprising a buffering agent has a pH ranging from about 3 to about 8.

In still other embodiments, any of the aforementioned compositions further comprise a humectant. In one embodiment, the humectant is sorbitol or glycerol. In further embodiments, any of the
15 aforementioned compositions further comprise a preservative. In one embodiment, the preservative comprises benzoic acid, benzyl alcohol, phenoxyethanol, methylparaben, propylparaben, or a combination thereof. As used herein, any reference to an acid may include a free acid, a salt, and any ester thereof. In other embodiments, any of the aforementioned compositions
20 further comprise a humectant and a preservative.

In certain embodiments, the antimicrobial cationic peptide is an indolicidin or an analog or derivative thereof in any one of the aforementioned compositions. In other embodiments, the cationic peptide is at a concentration ranging from about 0.01% to about 10% or from about 0.5% to about 1.5% in
25 any one of the aforementioned compositions. In yet other embodiments, any one of the aforementioned compositions having a cationic peptide that is a peptide of up to 35 amino acids, comprising one of the following sequences:
11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN,
30 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, 11J67CN, 11J68CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN.

In another aspect there is provided a composition comprising an antimicrobial cationic peptide, a viscosity-increasing agent, a solvent, a
35 humectant, and a buffering agent. In one embodiment, the humectant is sorbitol or glycerol. In certain embodiments, the buffering agent is at a

concentration ranging from about 1mM to about 200mM. In other embodiments, the buffering agent comprises a monocarboxylate or a dicarboxylate. In further embodiments, the buffering agent is acetate, fumarate, lactate, malonate, succinate, or tartrate. In yet another embodiment, the composition has a pH ranging from about 3 to about 8. In another embodiment, the composition further comprises a preservative. In one embodiment, the preservative comprises benzoic acid, benzyl alcohol, phenoxyethanol, methylparaben, propylparaben, or a combination thereof. In certain embodiments, the solvent is water, glycerin, propylene glycol, isopropanol, ethanol, or methanol. In certain other embodiments, the solvent is glycerin at a concentration ranging from about 0.1% to about 20% or from about 9% to about 11%. In still other embodiments, the solvent is propylene glycol at a concentration ranging from about 0.1% to about 20% or from about 9% to about 11%. In yet other embodiments, the solvent comprises at least one of water, glycerin, propylene glycol, isopropanol, ethanol, and methanol. In further embodiments, the solvent comprises at least one of water at a concentration up to 99%, glycerin at a concentration up to 20%, propylene glycol at a concentration up to 20%, ethanol at a concentration up to 99%, and methanol at a concentration up to 99%.

In certain embodiments, the viscosity-increasing agent is dextran, polyvinylpyrrolidone, hydroxyethyl cellulose, or hydroxypropyl methylcellulose. In another embodiment, the viscosity-increasing agent is hydroxyethyl cellulose at a concentration ranging from about 0.5% to about 5% or from about 1% to about 3%. In another embodiment, the viscosity-increasing agent is hydroxypropyl methylcellulose at a concentration ranging from about 1% to about 3%. In other embodiments, the viscosity-increasing agent is dextran at a concentration ranging from about 0.1% to about 5% or from about 0.5% to about 1%. In certain other embodiments, where the viscosity-increasing agent is hydroxyethyl cellulose, the composition further comprises a second viscosity-increasing agent of dextran, polyvinylpyrrolidone, or hydroxypropyl methylcellulose. In one embodiment, the second viscosity-increasing agent is polyvinylpyrrolidone. In related embodiments, the polyvinylpyrrolidone is at a concentration ranging from about 0.1% to about 5% or from about 0.5% to about 1%. In another embodiment, the second viscosity-increasing agent is hydroxypropyl methylcellulose. In related embodiments, the hydroxypropyl methylcellulose is at a concentration ranging from about 1% to about 3%. In

certain other embodiments, where the viscosity-increasing agent is hydroxypropyl methylcellulose, the composition further comprises a second viscosity-increasing agent of dextran, or dextran at concentration ranging from about 0.1% to about 5% or from about 0.5% to about 1%. In still other
5 embodiments, where the viscosity-increasing agent is hydroxypropyl methylcellulose, the composition further comprises a second viscosity-increasing agent of polyvinylpyrrolidone, or polyvinylpyrrolidone at a concentration ranging from about 0.1% to about 5% or from about 0.5% to about 1%.

10 In certain embodiments, the antimicrobial cationic peptide is an indolicidin or an analog or derivative thereof in any one of the aforementioned compositions. In other embodiments, the cationic peptide is at a concentration ranging from about 0.01% to about 10% or from about 0.5% to about 1.5% in any one of the aforementioned compositions. In yet other embodiments, the
15 cationic peptide is a peptide of up to 35 amino acids, comprising one of the following sequences: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, 11J67CN, 11J68CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN in any one of the aforementioned
20 compositions.

In still another aspect, the present invention provides a composition comprising an antimicrobial cationic peptide, a buffering agent, and a solvent. In other embodiments, the composition further comprises a
25 humectant. In one embodiment, the humectant is sorbitol or glycerol. In another embodiment, the composition further comprises a preservative. In one embodiment, the preservative comprises benzoic acid, benzyl alcohol, phenoxyethanol, methylparaben, propylparaben, or a combination thereof. In yet other embodiments, the composition further comprises a viscosity-
30 increasing agent of dextran, polyvinylpyrrolidone, hydroxyethyl cellulose, or hydroxypropyl methylcellulose. In another embodiment, the viscosity-increasing agent is hydroxyethyl cellulose at a concentration ranging from about 1% to about 3%. In another embodiment, the viscosity-increasing agent is hydroxypropyl methylcellulose at a concentration ranging from about 1% to
35 about 3%. In certain other embodiments, wherein the viscosity-increasing agent is hydroxyethyl cellulose, the composition further comprises a second

viscosity-increasing agent of hydroxypropyl methylcellulose. In another embodiment, the viscosity-increasing agent is hydroxyethyl cellulose at a concentration up to about 3% and the second viscosity-increasing agent is hydroxypropyl methylcellulose at a concentration up to about 3%. In a further
5 embodiment, the composition further comprises an acne medicament of retinoid, vitamin D3, or corticosteroid, and analogues or derivatives thereof.

In certain embodiments the solvent is water, glycerin, propylene glycol, isopropanol, ethanol, or methanol. In certain embodiments, the solvent is glycerin at a concentration ranging from about 9% to about 11%. In another
10 embodiment, the solvent is propylene glycol at a concentration ranging from about 9% to about 11%. In yet other embodiments, the solvent comprises at least one of water, glycerin, propylene glycol, isopropanol, ethanol, and methanol. In further embodiments, the solvent comprises at least one of water at a concentration up to 99%, glycerin at a concentration up to 20%, propylene
15 glycol at a concentration up to 20%, ethanol at a concentration up to 99%, and methanol at a concentration up to 99%.

In certain embodiments, the buffering agent comprises a monocarboxylate or a dicarboxylate. In other embodiments, the buffering agent is acetate, fumarate, lactate, malonate, succinate, or tartrate. In yet another
20 embodiment, the composition has a pH ranging from about 3 to about 8. In further embodiments, the buffering agent is at a concentration ranging from about 1mM to about 200mM or from about 4mM to about 6mM.

In certain embodiments, the antimicrobial cationic peptide is an indolicidin or an analog or derivative thereof in any one of the aforementioned
25 compositions. In other embodiments, the cationic peptide is at a concentration ranging from about 0.01% to about 10% or from about 0.5% to about 1.5% in any one of the aforementioned compositions. In yet other embodiments, the cationic peptide is a peptide of up to 35 amino acids, comprising one of the following sequences: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN,
30 11F5CN, 11F12CN, 11F17CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, 11J67CN, 11J68CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN in any one of the aforementioned compositions.

35 In yet another aspect, the present invention provides a composition comprising an antimicrobial cationic peptide at a concentration

ranging from about 0.01% to about 10%; a viscosity-increasing agent of dextran, polyvinylpyrrolidone, hydroxyethyl cellulose, or hydroxypropyl methylcellulose; and a solvent of water, glycerin, propylene glycol, isopropanol, ethanol, or methanol; at a pH ranging from about 3 to about 8. In certain
 5 embodiments, the composition comprises hydroxyethyl cellulose at a concentration ranging from about 1% to about 2%. In other embodiments, the composition comprises glycerin at a concentration ranging from about 9% to about 11%. In another embodiment, the composition further comprises a buffering agent. In one embodiment, the composition further comprising the
 10 buffering agent has a pH ranging from about 3.5 to about 7. In other embodiments, the buffering agent comprises a monocarboxylate or a dicarboxylate. In yet other embodiments, the buffering agent is acetate, fumarate, lactate, malonate, succinate, or tartrate. In certain other embodiments, the buffering agent is at a concentration ranging from about 1mM
 15 to about 200mM or from about 4mM to about 6mM. In another embodiment, the composition further comprises a preservative. In one embodiment, the preservative comprises benzoic acid, benzyl alcohol, phenoxyethanol, methylparaben, propylparaben, or a combination thereof.

In certain embodiments, the antimicrobial cationic peptide is an
 20 indolicidin or an analog or derivative thereof in any one of the aforementioned compositions. In other embodiments, the cationic peptide is a peptide of up to 35 amino acids, comprising one of the following sequences: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN,
 25 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, 11J67CN, 11J68CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN in any one of the aforementioned compositions.

In a further aspect, the present invention provides a composition comprising (a) an antimicrobial cationic peptide wherein the cationic peptide is
 30 a peptide of up to 35 amino acids comprising one of the following: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, 11J67CN, 11J68CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-
 35 11J38CN; (b) a viscosity-increasing agent wherein the viscosity-increasing agent is hydroxyethyl cellulose at a concentration of about 1.2% to about 1.8%;

(c) a buffer wherein the buffer is lactate at a concentration ranging from about 4mM to about 6mM; (d) a solvent wherein the solvent comprises glycerin at a concentration ranging from about 9% to about 11% and water at a concentration ranging from about 85% to about 90%; and (e) a pH ranging from about 3.5 to about 7. In certain embodiments, the cationic peptide is at a concentration ranging from about 0.8% to about 1.2%. In yet another embodiment, provided are methods to reduce microflora, or to treat or prevent an infection, at a target site, the target site may be skin, and the skin may further comprise acne. In another embodiment, the composition may be applied to a target site to treat or prevent or ameliorate inflammation, such as inflammation associated with acne (or with an implanted or indwelling medical device).

Another aspect of the present invention is a composition, comprising (a) an antimicrobial cationic peptide wherein the cationic peptide is a peptide of up to 35 amino acids comprising one of the following: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, 11J67CN, 11J68CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN; (b) a buffer wherein the buffer is lactate at a concentration ranging from about 4mM to about 6mM; (c) a solvent wherein the solvent comprises ethanol at a concentration ranging from about 45% to about 55% and water at a concentration ranging from about 44% to about 54%; and (d) a pH ranging from about 3.5 to about 7. In certain embodiments, the cationic peptide is at a concentration ranging from about 0.8% to about 1.2%. In other embodiments, the composition may further comprise an acne medicament such as retinoid, vitamin D3, or corticosteroid, and analogs or derivatives thereof. In yet another embodiment, provided are methods to reduce microflora, or to treat or prevent an infection, at a target site, the target site may be skin, and the skin may further comprise acne. In another embodiment, the composition may be applied to a target site to treat or prevent or ameliorate inflammation, such as inflammation associated with acne (or with an implanted or indwelling medical device).

In still another aspect, the present invention provides a composition comprising (a) an antimicrobial cationic peptide wherein the cationic peptide is a peptide of up to 35 amino acids comprising one of the

following: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, 11J67CN, 11J68CN, Nt-acryloyl-11B7CN, Nt-glucosyl-
5 11J36CN, or Nt-glucosyl-11J38CN; (b) a viscosity-increasing agent wherein the viscosity-increasing agent is hydroxyethyl cellulose at a concentration of about 1.2% to about 1.8%; (c) a solvent wherein the solvent comprises glycerin at a concentration ranging from about 9% to about 11% and water at a concentration ranging from about 85% to about 90%; (d) a preservative wherein
10 the preservative is benzoic acid at a concentration ranging from about 20mM to about 30mM; and (e) a pH ranging from about 3.5 to about 4.7. In certain embodiments, the cationic peptide is at a concentration ranging from about 0.8% to about 1.2%, or ranging from about 2.5% to about 3.5%. In yet another embodiment, provided are methods to reduce microflora, or to treat or prevent
15 an infection, at a target site, the target site may be skin, and the skin may further comprise acne. In another embodiment, the composition may be applied to a target site to treat or prevent or ameliorate inflammation, such as inflammation associated with acne (or with an implanted or indwelling medical device).
20 In another aspect there is provided a method for reducing microflora at a target site, comprising applying to the target site a composition comprising an antimicrobial cationic peptide, a viscosity-increasing agent, and a solvent. In certain embodiments, the microflora is a prokaryotic organism, a eukaryotic organism, or a virus. In some embodiments the target site is skin
25 and in others the skin further comprises acne. In other embodiments, the target site is a mucosa, and in still other embodiments the mucosa further comprises a nasal passage. In one embodiment the nasal passage is an anterior naris. In certain other embodiments, the method further comprises inserting a medical device at the target site before or after applying the composition. In yet another
30 embodiment, the method further comprises applying the composition to the device prior to inserting the device at the target site. In one embodiment, the device comprises a catheter and another embodiment is a central venous catheter. In certain other embodiments, the catheter is a vascular dialysis catheter, a pulmonary artery catheter, a peritoneal dialysis catheter, or an
35 umbilical catheter.

In a further aspect, the present invention provides a method for treating or preventing infection at a target site, comprising applying to the target site a composition comprising a cationic peptide, a viscosity-increasing agent, and a solvent. In certain embodiments, the infection is caused by a prokaryotic organism, a eukaryotic organism, or a virus. In other embodiments, the infection at a target site is associated with a medical device at the target site. In further embodiments, the method comprises applying the composition prior to or after inserting a medical device at the target. In one embodiment, the device comprises a catheter and another embodiment is a central venous catheter. In certain other embodiments, the catheter is a vascular dialysis catheter, a pulmonary artery catheter, a peritoneal dialysis catheter, or an umbilical catheter. In some embodiments the target site is skin and in others the skin further comprises acne. In other embodiments, the target site is a mucosa, and in still other embodiments the mucosa further comprises a nasal passage. In one embodiment the nasal passage is an anterior naris.

In yet another aspect there is provided a method treating or preventing inflammation at a target site, comprising applying to the target site a composition comprising a cationic peptide, a viscosity-increasing agent, and a solvent. In one embodiment, the target site further comprises an infection. In a further embodiment, the inflammation at the target site is associated with a medical device. In further embodiments, the method comprises applying the composition prior to or after inserting a medical device at the target. In one embodiment, the device comprises a catheter and another embodiment is a central venous catheter. In certain other embodiments, the catheter is a vascular dialysis catheter, a pulmonary artery catheter, a peritoneal dialysis catheter, or an umbilical catheter. In some embodiments the target site is skin and in others the skin further comprises acne. In other embodiments, the target site is a mucosa, and in still other embodiments the mucosa further comprises a nasal passage. In one embodiment the nasal passage is an anterior naris.

In yet another aspect, the invention provides a method for ameliorating inflammation at a target site, comprising applying to the target site a composition comprising a cationic peptide, a viscosity-increasing agent, and a solvent. In one embodiment, the target site further comprises an infection. In a further embodiment, the inflammation at the target site is associated with a medical device. In further embodiments, the method comprises applying the composition prior to or after inserting a medical device at the target. In one

embodiment, the device comprises a catheter and another embodiment is a central venous catheter. In certain other embodiments, the catheter is a vascular dialysis catheter, a pulmonary artery catheter, a peritoneal dialysis catheter, or an umbilical catheter. In some embodiments the target site is skin and in others the skin further comprises acne. In other embodiments, the target site is a mucosa, and in still other embodiments the mucosa further comprises a nasal passage. In one embodiment the nasal passage is an anterior naris.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, various references are set forth herein which describe in more detail certain procedures or compositions, and are therefore incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figure 1 shows the results of a bacterial challenge test of an antimicrobial cationic peptide (1%, gel) formulation, illustrating the antimicrobial effectiveness of the formulation when seeded with a large inoculum of various bacteria and fungi.

Figure 2 shows the results of a bacterial challenge test of an antimicrobial cationic peptide (1%, petrolatum) formulation illustrating the antimicrobial effectiveness of the formulation.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention provides compositions and methods for using antimicrobial cationic peptides to treat and/or prevent infectious diseases. The invention, therefore, relates generally to the surprising discovery that antimicrobial cationic peptides may be formulated at a clinically relevant concentration to maximize their *in vivo* and *in vitro* stability, release half-life, and antimicrobial activity. According to the present invention, formulations of antimicrobial cationic peptides (e.g., indolicidins and derivatives or analogs thereof), as described herein, provide novel and useful compositions for use in a variety of therapeutic settings (e.g., in the treatment and prevention of nosocomial infections, acne, and infections associated with intravascular penetration, such as in the use of hypodermic needles and catheters). Discussed in more detail below are cationic peptides suitable for use within the

present invention, as well as representative formulations and therapeutic uses. Any concentration ranges recited herein are to be understood to include concentrations of any integer within the range and fractions thereof, such as one tenth and one hundredth of an integer, unless otherwise indicated.

A. ANTIMICROBIAL CATIONIC PEPTIDES

The present invention is directed generally to antimicrobial cationic peptides, which may be produced by a variety of methods (e.g., chemical or recombinant) for use in the formulations as described herein.

10 Suitable antimicrobial cationic peptides include, but are not limited to, naturally occurring cationic peptides, which have been isolated, and derivatives or analogs thereof. An "isolated peptide, polypeptide, or protein" is an amino acid sequence that is essentially free from contaminating cellular components, such as carbohydrate, lipid, nucleic acid (DNA or RNA), or other proteinaceous

15 impurities associated with the polypeptide in nature. Preferably, the isolated polypeptide is sufficiently pure for therapeutic use at the desired dose.

An antimicrobial cationic peptide of the present invention may be a recombinant peptide or a synthetic peptide, and is preferably a recombinant peptide. Peptides may be synthesized by standard chemical methods,

20 including synthesis by automated procedure. In general, peptide analogues are synthesized based on the standard solid-phase Fmoc protection strategy with HATU as the coupling agent. The peptide is cleaved from the solid-phase resin with trifluoroacetic acid containing appropriate scavengers, which also deprotects side chain functional groups. Crude peptide is further purified using

25 preparative reversed-phase chromatography. Other purification methods, such as partition chromatography, gel filtration, gel electrophoresis, or ion-exchange chromatography may be used. Other synthesis techniques, known in the art, such as the tBoc protection strategy, or use of different coupling reagents or the like can be employed to produce equivalent peptides. Peptides may be

30 synthesized as a linear molecule or as branched molecules. Branched peptides typically contain a core peptide that provides a number of attachment points for additional peptides. Lysine is most commonly used for the core peptide because it has one carboxyl functional group and two (alpha and epsilon) amine functional groups. Other diamino acids can also be used.

35 Preferably, either two or three levels of geometrically branched lysines are

used; these cores form a tetrameric and octameric core structure, respectively (Tam, *Proc. Natl. Acad. Sci. USA* 85:5409, 1988).

An antimicrobial cationic peptide is a peptide that typically exhibits a positive charge at a pH ranging from about 3 to about 10 (*i.e.*, has an isoelectric point of at least about 9), and contains at least one basic amino acid (e.g., arginine, lysine, histidine). In addition, an antimicrobial cationic peptide generally comprises an amino acid sequence having a molecular mass of about 0.5 kDa (*i.e.*, approximately five amino acids in length) to about 10 kDa (*i.e.*, approximately 100 amino acids in length), or a molecular mass of any integer, or fraction thereof (including a tenth and one hundredth of an integer), ranging from about 0.5 kDa to about 10 kDa. Preferably, an antimicrobial cationic peptide has a molecular mass ranging from about 0.5 kDa to about 5 kDa (*i.e.*, approximately from about 5 amino acids to about 45 amino acids in length), more preferably from about 1 kDa to about 4 kDa (*i.e.*, approximately from about 10 amino acids to about 35 amino acids in length), and most preferably from about 1 kDa to about 2 kDa (*i.e.*, approximately from about 10 amino acids to about 18 amino acids in length). In another preferred embodiment, the antimicrobial cationic peptide is part of a larger peptide or polypeptide sequence having, for example, a total of up to 100 amino acids, more preferably up to 50 amino acids, even more preferably up to 35 amino acids, and most preferably up to 15 amino acids. The present invention contemplates an antimicrobial cationic peptide having an amino acid sequence of 5 to 100 amino acids, with the number of amino acids making up the peptide sequence comprising any integer in that range. An antimicrobial cationic peptide may exhibit antibacterial activity, anti-endotoxin activity, antifungal activity, antiparasite activity, antiviral activity, anticancer activity, anti-inflammatory activity, wound healing activity, and synergistic activity with other peptides or antimicrobial compounds, or a combination thereof.

Exemplary antimicrobial peptides include, but are not limited to, cecropins, normally made by lepidoptera (Steiner *et al.*, *Nature* 292:246, 1981) and diptera (Merrifield *et al.*, *Ciba Found. Symp.* 186:5, 1994), by porcine intestine (Lee *et al.*, *Proc. Nat'l Acad. Sci. USA* 86:9159, 1989), by blood cells of a marine protochordate (Zhao *et al.*, *FEBS Lett.* 412:144, 1997); synthetic analogs of cecropin A, melittin, and cecropin-melittin chimeric peptides (Wade *et al.*, *Int. J. Pept. Protein Res.* 40:429, 1992); cecropin B analogs (Jaynes *et al.*, *Plant Sci.* 89:43, 1993); chimeric cecropin A/B hybrids (Düring, *Mol. Breed.*

2:297, 1996); magainins (Zasloff, *Proc. Nat'l Acad. Sci USA* 84:5449, 1987); cathelin-associated antimicrobial peptides from leukocytes of humans, cattle, pigs, mice, rabbits, and sheep (Zanetti *et al.*, *FEBS Lett.* 374:1, 1995); vertebrate defensins, such as human neutrophil defensins [HNP 1-4]; paneth cell defensins of mouse and human small intestine (Oulette and Selsted, *FASEB J.* 10:1280, 1996; Porter *et al.*, *Infect. Immun.* 65:2396, 1997); vertebrate β -defensins, such as HBD-1 of human epithelial cells (Zhao *et al.*, *FEBS Lett.* 368:331, 1995); HBD-2 of inflamed human skin (Harder *et al.*, *Nature* 387:861, 1997); bovine β -defensins (Russell *et al.*, *Infect. Immun.* 64:1565, 1996); plant defensins, such as Rs-AFP1 of radish seeds (Fehlbaum *et al.*, *J. Biol. Chem.* 269:33159, 1994); α - and β -thionins (Stuart *et al.*, *Cereal Chem.* 19:288, 1942; Bohlmann and Apel, *Annu. Rev. Physiol. Plant Mol. Biol.* 42:227, 1991); γ -thionins (Broekaert *et al.*, *Plant Physiol.* 108:1353, 1995); the anti-fungal drosomycin (Fehlbaum *et al.*, *J. Biol. Chem.* 269:33159, 1994); apidaecins, produced by honey bee, bumble bee, cicada killer, hornet, yellow jacket, and wasp (Casteels *et al.*, *J. Biol. Chem.* 269:26107, 1994; Levashina *et al.*, *Eur. J. Biochem.* 233:694, 1995); cathelicidins, such as indolicidin and derivatives or analogues thereof from bovine neutrophils (Falla *et al.*, *J. Biol. Chem.* 277:19298, 1996); bacteriocins, such as nisin (Delves-Broughton *et al.*, *Antonie van Leeuwenhoek J. Microbiol.* 69:193, 1996); and the protegrins and tachyplesins, which have antifungal, antibacterial, and antiviral activities (Tamamura *et al.*, *Biochim. Biophys. Acta* 1163:209, 1993; Aumelas *et al.*, *Eur. J. Biochem.* 237:575, 1996; Iwanga *et al.*, *Ciba Found. Symp.* 186:160, 1994).

In certain embodiments, preferred antimicrobial cationic peptides of the present invention are indolicidins or analogs or derivatives thereof (see Table 2 of Example 1). Natural indolicidins may be isolated from a variety of organisms, and, for example, the indolicidin isolated from bovine neutrophils is a 13 amino acid peptide, which is tryptophan-rich and amidated at the C-terminus (see Selsted *et al.*, *J. Biol. Chem.* 267:4292, 1992). As noted above, a preferred indolicidin or analog or derivative thereof comprises 5 to 45 amino acids, more preferably 7 to 35 amino acids, even more preferably 8 to 25 amino acids, and most preferably 10 to 14 amino acids (see, e.g., Table 2). The indolicidins or analogs or derivatives thereof of the present invention may be used at a concentration ranging from about 0.01% to about 10%, preferably from about 0.5% to about 5%, and more preferably from either about 1% to about 3% or about 4% to about 6%, depending on the intended use and

formulation ingredients (where "about" is $\pm 10\%$ of the indicated value). In certain embodiments, the antimicrobial cationic peptide is an indolicidin or an analog or derivative thereof in any one of the aforementioned compositions. In preferred embodiments, the antimicrobial cationic peptide is a peptide of up to
5 35 amino acids, comprising one of the following sequences: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, 11J67CN, 11J68CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-
10 11J38CN, which may be used in any one of the compositions described herein.

An antimicrobial cationic peptide of the present invention may be an analog or derivative thereof. As used herein, the terms "derivative" and "analog" when referring to an antimicrobial cationic peptide, polypeptide, or fusion protein, refer to any antimicrobial cationic peptide, polypeptide, or fusion
15 protein that retain essentially the same (at least 50%, and preferably greater than 70, 80, or 90%) or enhanced biological function or activity as such natural peptide, as noted above. The biological function or activity of such analogs and derivatives can be determined using standard methods (e.g., antimicrobial, anti-inflammatory, DNA and/or protein synthesis inhibitor), such as with the assays
20 described herein. For example, an analog or derivative may be a proprotein that can be activated by cleavage to produce an active antimicrobial cationic peptide. Alternatively, a cationic peptide analog or derivative thereof can be identified by the ability to specifically bind anti-cationic peptide antibodies.

A cationic peptide analog or derivative may have, for example,
25 one or more deletion, insertion, or modification of any amino acid residue, including the N- or C-terminal amino acids. Within the scope of this invention are modified antimicrobial cationic peptides, such as, for example, peptides having an acetylated, acylated, acryloylated, alkylated, glycosylated (e.g., glucosylated), PEGylated, myristylated, and the like N-terminal amino acid
30 modification; having an esterified, amidated, homoserine/homoserine lactone, or caprolactam C-terminal amino acid modification; or having a polyalkylene glycol (e.g., polyethylene glycol) conjugated to any free amino group. A preferred modification of the C-terminal amino acid is amidation.

An analog or derivative may also be an antimicrobial cationic
35 peptide fusion protein. Fusion proteins, or chimeras, include fusions of one or more antimicrobial cationic peptides, and fusions of cationic peptides with non-

cationic peptides. Additionally, the peptide may be modified to form a polymer-modified peptide. The peptides may also be labeled, such as with a radioactive label, a fluorescent label, a mass spectrometry tag, biotin, and the like.

Another example of an analog or derivative includes an antimicrobial cationic peptide that has one or more conservative amino acid substitutions, as compared with the amino acid sequence of a naturally occurring cationic peptide. Among the common amino acids, a "conservative amino acid substitution" is illustrated, for example, by a substitution among amino acids within each of the following groups: (1) glycine, alanine, valine, leucine, and isoleucine, (2) phenylalanine, tyrosine, and tryptophan, (3) serine and threonine, (4) aspartate and glutamate, (5) glutamine and asparagine, and (6) lysine, arginine and histidine, or a combination thereof. Furthermore, an analog or derivative of a cationic peptide may include, for example, non-protein amino acids, such as precursors of normal amino acids (e.g., homoserine and diaminopimelate), intermediates in catabolic pathways (e.g., pipecolic acid and D-enantiomers of normal amino acids), and amino acid analogs (e.g., azetidine-2-carboxylic acid and canavanine).

The amino acid designations are herein set forth as either the standard one- or three-letter code. Unless otherwise indicated, a named amino acid refers to the L-enantiomer. Polar amino acids include asparagine (Asp or N) and glutamine (Gln or Q); as well as basic amino acids such as arginine (Arg or R), lysine (Lys or K), histidine (His or H), and derivatives thereof; and acidic amino acids such as aspartic acid (Asp or D) and glutamic acid (Glu or E), and derivatives thereof. Hydrophobic amino acids include tryptophan (Trp or W), phenylalanine (Phe or F), isoleucine (Ile or I), leucine (Leu or L), methionine (Met or M), valine (Val or V), and derivatives thereof; as well as other non-polar amino acids such as glycine (Gly or G), alanine (Ala or A), proline (Pro or P), and derivatives thereof. Amino acids of intermediate polarity include serine (Ser or S), threonine (Thr or T), tyrosine (Tyr or Y), cysteine (Cys or C), and derivatives thereof. A capital letter indicates an L-enantiomer amino acid; a small letter indicates a D-enantiomer amino acid. For example, some modified amino acids may include 2,3-diamino butyric acid, 3- or 4-mercaptoproline derivatives, N⁵-acetyl-N⁵-hydroxy-L-ornithine, and α -N-hydroxyamino acids. An antimicrobial cationic peptide analog or derivative thereof may include any one or combination of the above-noted alterations to the natural peptide, or any other modification known in the art.

The indolicidins or analogs or derivatives thereof of the present invention may be used individually, or may be used in combination with one or more different indolicidins or analogs or derivatives thereof, with one or more antimicrobial-cationic peptides, and one or more conventional antimicrobial agents, as described herein. Thus, synergistic combinations of an antimicrobial cationic peptide and an antimicrobial agent may permit a reduction in the dosage of one or both agents in order to achieve a similar or improved therapeutic effect. This would allow the use of smaller doses and, therefore, would decrease the potential incidence of toxicity (e.g., from aminoglycosides) and lowering costs of expensive antimicrobials (e.g., vancomycin). Concurrent or sequential administration of an antimicrobial cationic peptide formulation and an antimicrobial agent composition is expected to provide more effective treatment of infections caused by a variety of microorganisms (e.g., bacteria, viruses, fungi, and parasites). In particular, successful treatment or prevention of infectious disease can be achieved by using the antimicrobial cationic peptides and antimicrobial agents at doses below what is normally a therapeutically effective dose when these antimicrobials are used individually. Alternatively, the antibiotic agent and antimicrobial cationic peptide formulation can be administered using a normally effective therapeutic dose for each antimicrobial, but wherein the combination of the two agents provides even more potent effects.

As noted above, the preferred antimicrobial cationic peptides may be used in a synergistic combination with other known antimicrobial agents. Antibacterial agents include, but are not limited to, penicillins, cephalosporins, carbacephems, cephamycins, carbapenems, monobactams, aminoglycosides, glycopeptides, quinolones, tetracyclines, macrolides, and fluoroquinolones. Examples of antibiotic agents include, but are not limited to, Penicillin G (CAS Registry No.: 61-33-6); Methicillin (CAS Registry No.: 61-32-5); Nafcillin (CAS Registry No.: 147-52-4); Oxacillin (CAS Registry No.: 66-79-5); Cloxacillin (CAS Registry No.: 61-72-3); Dicloxacillin (CAS Registry No.: 3116-76-5); Ampicillin (CAS Registry No.: 69-53-4); Amoxicillin (CAS Registry No.: 26787-78-0); Ticarcillin (CAS Registry No.: 34787-01-4); Carbenicillin (CAS Registry No.: 4697-36-3); Mezlocillin (CAS Registry No.: 51481-65-3); Azlocillin (CAS Registry No.: 37091-66-0); Piperacillin (CAS Registry No.: 61477-96-1); Imipenem (CAS Registry No.: 74431-23-5); Aztreonam (CAS Registry No.: 78110-38-0); Cephalothin (CAS Registry No.: 153-61-7); Cefazolin (CAS

- Registry No.: 25953-19-9); Cefaclor (CAS Registry No.: 70356-03-5); Cefamandole formate sodium (CAS Registry No.: 42540-40-9); Cefoxitin (CAS Registry No.: 35607-66-0); Cefuroxime (CAS Registry No.: 55268-75-2); Cefonicid (CAS Registry No.: 61270-58-4); Cefmetazole (CAS Registry No.: 56796-20-4); Cefotetan (CAS Registry No.: 69712-56-7); Cefprozil (CAS Registry No.: 92665-29-7); Lincomycin (CAS Registry No.: 154-21-2); Linezolid (CAS Registry No.: 165800-03-3); Loracarbef (CAS Registry No.: 121961-22-6); Cefetamet (CAS Registry No.: 65052-63-3); Cefoperazone (CAS Registry No.: 62893-19-0); Cefotaxime (CAS Registry No.: 63527-52-6); Ceftizoxime (CAS Registry No.: 68401-81-0); Ceftriaxone (CAS Registry No.: 73384-59-5); Ceftazidime (CAS Registry No.: 72558-82-8); Cefepime (CAS Registry No.: 88040-23-7); Cefixime (CAS Registry No.: 79350-37-1); Cefpodoxime (CAS Registry No.: 80210-62-4); Cefsulodin (CAS Registry No.: 62587-73-9); Fleroxacin (CAS Registry No.: 79660-72-3); Nalidixic acid (CAS Registry No.: 389-08-2); Norfloxacin (CAS Registry No.: 70458-96-7); Ciprofloxacin (CAS Registry No.: 85721-33-1); Ofloxacin (CAS Registry No.: 82419-36-1); Enoxacin (CAS Registry No.: 74011-58-8); Lomefloxacin (CAS Registry No.: 98079-51-7); Cinoxacin (CAS Registry No.: 28657-80-9); Doxycycline (CAS Registry No.: 564-25-0); Minocycline (CAS Registry No.: 10118-90-8); Tetracycline (CAS Registry No.: 60-54-8); Amikacin (CAS Registry No.: 37517-28-5); Gentamicin (CAS Registry No.: 1403-66-3); Kanamycin (CAS Registry No.: 8063-07-8); Netilmicin (CAS Registry No.: 56391-56-1); Tobramycin (CAS Registry No.: 32986-56-4); Streptomycin (CAS Registry No.: 57-92-1); Azithromycin (CAS Registry No.: 83905-01-5); Clarithromycin (CAS Registry No.: 81103-11-9); Erythromycin (CAS Registry No.: 114-07-8); Erythromycin estolate (CAS Registry No.: 3521-62-8); Erythromycin ethyl succinate (CAS Registry No.: 41342-53-4); Erythromycin glucoheptonate (CAS Registry No.: 23067-13-2); Erythromycin lactobionate (CAS Registry No.: 3847-29-8); Erythromycin stearate (CAS Registry No.: 643-22-1); Vancomycin (CAS Registry No.: 1404-90-6); Teicoplanin (CAS Registry No.: 61036-64-4); Chloramphenicol (CAS Registry No.: 56-75-7); Clindamycin (CAS Registry No.: 18323-44-9); Trimethoprim (CAS Registry No.: 738-70-5); Sulfamethoxazole (CAS Registry No.: 723-46-6); Nitrofurantoin (CAS Registry No.: 67-20-9); Rifampin (CAS Registry No.: 13292-46-1); Mupirocin (CAS Registry No.: 12650-69-0); Metronidazole (CAS Registry No.: 443-48-1); Cephalixin (CAS Registry No.: 15686-71-2); Roxithromycin (CAS Registry No.: 80214-83-1); Co-

amoxiclavuanate; combinations of Piperacillin and Tazobactam; and their various salts, acids, bases, and other derivatives.

The antimicrobial cationic peptide may also be used in combination with anti-fungal agents. Exemplary anti-fungal agents include, but are not limited to, terbinafine hydrochloride, nystatin, amphotericin B, griseofulvin, ketoconazole, miconazole nitrate, flucytosine, fluconazole, itraconazole, clotrimazole, benzoic acid, salicylic acid, and selenium sulfide.

The antimicrobial cationic peptide may also be used in combination with anti-viral agents. Exemplary anti-viral agents include, but are not limited to, amantadine hydrochloride, rimantadin, acyclovir, famciclovir, foscarnet, ganciclovir sodium, idoxuridine, ribavirin, sorivudine, trifluridine, valacyclovir, vidarabin, didanosine, stavudine, zalcitabine, zidovudine, interferon alpha, and edoxudine.

The antimicrobial cationic peptide may also be used in combination with anti-parasitic agents. Exemplary anti-parasitic agents include, but are not limited to, pyrethrins/piperonyl butoxide, permethrin, iodoquinol, metronidazole, diethylcarbamazine citrate, piperazine, pyrantel pamoate, mebendazole, thiabendazole, praziquantel, albendazole, proguanil, quinidine gluconate injection, quinine sulfate, chloroquine phosphate, mefloquine hydrochloride, primaquine phosphate, atovaquone, co-trimoxazole (sulfamethoxazole/trimethoprim), and pentamidine isethionate.

In another aspect, antibodies may be generated to a specific antimicrobial cationic peptide and analogue or derivative thereof using multiple antigenic peptides (MAPs) that contain approximately eight copies of the peptide linked to a small non-immunogenic peptidyl core to form an immunogen (see, in general, *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988). Alternatively, the target peptide may be conjugated to bovine serum albumin (BSA), ovalbumin, or another suitable conjugate. The MAP or peptide conjugate is injected subcutaneously into rabbits or into mice or other rodents, where they may have sufficiently long half-lives to facilitate antibody production. After twelve weeks, blood samples are taken and serum is separated for testing in an ELISA assay against the original peptide, with a positive result indicating the presence of antibodies specific to the target peptide. This serum can then be stored and used in ELISA assays to specifically measure the amount of the specific antimicrobial cationic peptide and/or analog or derivative thereof. Alternatively, other

standard methods of antibody production may be employed, for example generation of monoclonal antibodies.

Within the context of the present invention, antibodies are understood to include, *inter alia*, monoclonal antibodies, polyclonal antibodies, anti-idiotypic antibodies, and antibody fragments (e.g., Fab, and F(ab')₂, F_V variable regions, or complementarity determining regions). Antibodies are generally accepted as specific against cationic peptides, such as indolicidin and analogs or derivatives thereof, if they bind with a K_D of greater than or equal to 10⁻⁷ M, preferably greater than or equal to 10⁻⁸ M, and more preferably greater than of equal to 10⁻⁹ M. The affinity of a monoclonal antibody or binding partner may be readily determined by one of ordinary skill in the art (see, e.g., Scatchard, *Ann. N.Y. Acad. Sci.* 51:660-672, 1949). Once suitable antibodies have been identified, they may be isolated or purified by many techniques well known to those of ordinary skill in the art.

Monoclonal antibodies may also be readily generated from hybridoma cell lines using conventional techniques (see U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; see also *Antibodies: A Laboratory Manual*, 1988). Briefly, within one embodiment, a subject animal, such as a rat or mouse, is injected with a peptide of choice. The peptide is generally administered in an emulsion with an adjuvant, such as Freund's complete or incomplete adjuvant, which is intended to increase the immune response. The animal is generally boosted at least once prior to harvest of the spleen and/or lymph nodes and immortalization of those cells. Various immortalization techniques, such as mediated by Epstein-Barr virus or fusion to produce a hybridoma, may be used. In a preferred embodiment, immortalization occurs by fusion with a suitable myeloma cell line to create a hybridoma that secretes monoclonal antibody. Suitable myeloma lines include, for example, NS-1 (ATCC No. TIB 18), and P3X63 - Ag 8.653 (ATCC No. CRL 1580). The preferred fusion partners do not express endogenous antibody genes. After about seven days, the hybridomas may be screened for the presence of antibodies that are reactive against an antimicrobial cationic peptide and analog or derivative thereof. A wide variety of assays may be utilized (see *Antibodies: A Laboratory Manual*, 1988).

Other techniques known in the art may be utilized to construct monoclonal antibodies (see Huse *et al.*, *Science* 246:1275-1281, 1989; Sastry *et al.*, *Proc. Natl. Acad. Sci. USA* 86:5728-5732, 1989; Alting-Mees *et al.*,

Strategies in Molecular Biology 3:1-9, 1990; describing recombinant techniques). These techniques include cloning heavy and light chain immunoglobulin cDNA in suitable vectors, such as λ ImmunoZap(H) and λ ImmunoZap(L). These recombinants may be screened individually or co-expressed to form Fab fragments or antibodies (see Huse *et al.*, *supra*; Sastry *et al.*, *supra*). Positive plaques may subsequently be converted into non-lytic plasmids to allow high-level expression of monoclonal antibody fragments in a host, such as *E. coli*.

Similarly, portions or fragments of antibodies, such as Fab and Fv fragments, may also be constructed utilizing conventional enzymatic digestion or recombinant DNA techniques to yield isolated variable regions of an antibody. Within one embodiment, the genes that encode the variable region from a hybridoma producing a monoclonal antibody of interest are amplified using nucleotide primers for the variable region. In addition, techniques may be utilized to change a "murine" antibody to a "human" antibody, without altering the binding specificity of the antibody to the antimicrobial cationic peptide and analog or derivative thereof.

B. NUCLEIC ACIDS ENCODING ANTIMICROBIAL CATIONIC PEPTIDES

Nucleic acid molecules encoding cationic peptides may be isolated from natural sources, may be obtained by automated synthesis of nucleic acid molecules, or may be obtained by using the polymerase chain reaction (PCR) with oligonucleotide primers having nucleotide sequences that are based upon known nucleotide sequences of antimicrobial cationic peptide genes. In the latter approach, a cationic peptide gene is synthesized using mutually priming oligonucleotides (see, for example, Ausubel *et al.* (eds.), *Short Protocols in Molecular Biology*, 3rd Edition, pages 8-8 to 8-9, John Wiley & Sons, 1995, herein after referred to as "Ausubel (1995)"). Established techniques using the polymerase chain reaction provide the ability to synthesize DNA molecules of at least two kilobases in length (Adang *et al.*, *Plant Molec. Biol.* 21:1131, 1993; Bambot *et al.*, *PCR Methods and Applications* 2:266, 1993; Dillon *et al.*, "Use of the Polymerase Chain Reaction for the Rapid Construction of Synthetic Genes," in *Methods in Molecular Biology*, Vol. 15: *PCR Protocols: Current Methods and Applications*, White (ed.), pages 263-268, Humana Press, Inc., 1993; Holowachuk *et al.*, *PCR Methods Appl.* 4:299, 1995).

As noted above, the present invention contemplates analogs or derivatives of natural cationic peptides, which analogs or derivatives may be recombinantly produced by the presently described methods. Nucleotide sequences encoding conservative amino acid analogs or derivatives can be
5 obtained, for example, by oligonucleotide-directed mutagenesis, linker-scanning mutagenesis, mutagenesis using the polymerase chain reaction, and the like (see Ausubel, 1995, at page 8-10 through page 8-22; and McPherson (ed.), *Directed Mutagenesis: A Practical Approach*, IRL Press, 1991).

Although one objective in constructing a cationic peptide variant
10 may be to improve its activity, it may also be desirable to alter the amino acid sequence of a naturally occurring cationic peptide to enhance its production in a recombinant host cell. The presence of a particular codon may have an adverse effect on expression in a particular host; therefore, a DNA sequence encoding the desired cationic peptide is optimized for a particular host system,
15 such as prokaryotic or eukaryotic cells. For example, a nucleotide sequence encoding a radish cationic peptide may include a codon that is commonly found in radish, but is rare for *E. coli*. The presence of a rare codon may have an adverse effect on protein levels when the radish cationic peptide is expressed in recombinant *E. coli*. Methods for altering nucleotide sequences to alleviate
20 the codon usage problem are well known to those of skill in the art (see, e.g., Kane, *Curr. Opin. Biotechnol.* 6:494, 1995; Makrides, *Microbiol. Rev.* 60:512, 1996; and Brown (Ed.), *Molecular Biology LabFax*, BIOS Scientific Publishers, Ltd., 1991, which provides a Codon Usage Table at page 245 through page 253).

25 Peptides may be synthesized by recombinant techniques (see e.g., U.S. Patent No. 5,593,866) and a variety of host systems are suitable for production of the cationic peptides and analogues or derivatives thereof, including bacteria (e.g., *E. coli*), yeast (e.g., *Saccharomyces cerevisiae*), insect (e.g., Sf9), and mammalian cells (e.g., CHO, COS-7). Many expression vectors
30 have been developed and are available for each of these hosts. Generally, vectors that are functional in bacteria are used in this invention. However, at times, it may be preferable to have vectors that are functional in other hosts. Vectors and procedures for cloning and expression in *E. coli* are discussed herein and, for example, in Sambrook et al. (*Molecular Cloning: A Laboratory*
35 *Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1987) and in Ausubel et al., 1995.

A DNA sequence encoding a cationic peptide is introduced into an expression vector appropriate for the host. In preferred embodiments, the gene is cloned into a vector to create a fusion protein. The fusion partner is chosen to contain an anionic region, such that a bacterial host is protected from the toxic effect of the peptide. This protective region effectively neutralizes the antimicrobial effects of the peptide and also may prevent peptide degradation by host proteases. The fusion partner (carrier protein) of the invention may further function to transport the fusion peptide to inclusion bodies, the periplasm, the outer membrane, or the extracellular environment. Carrier proteins suitable in the context of this invention specifically include, but are not limited to, glutathione-S-transferase (GST), protein A from *Staphylococcus aureus*, two synthetic IgG-binding domains (ZZ) of protein A, outer membrane protein F, β -galactosidase (*lacZ*), and various products of bacteriophage λ and bacteriophage T7. From the teachings provided herein, it is apparent that other proteins may be used as carriers. Furthermore, the entire carrier protein need not be used, as long as the protective anionic region is present. To facilitate isolation of the peptide sequence, amino acids susceptible to chemical cleavage (e.g., CNBr) or enzymatic cleavage (e.g., V8 protease, trypsin) are used to bridge the peptide and fusion partner. For expression in *E. coli*, the fusion partner is preferably a normal intracellular protein that directs expression toward inclusion body formation. In such a case, following cleavage to release the final product, there is no requirement for renaturation of the peptide. In the present invention, the DNA cassette, comprising fusion partner and peptide gene, may be inserted into an expression vector, which can be a plasmid, virus or other vehicle known in the art. Preferably, the expression vector is a plasmid that contains an inducible or constitutive promoter to facilitate the efficient transcription of the inserted DNA sequence in the host. Transformation of the host cell with the recombinant DNA may be carried out by Ca^{++} -mediated techniques, by electroporation, or other methods well known to those skilled in the art.

Briefly, a DNA fragment encoding a peptide is derived from an existing cDNA or genomic clone or synthesized. A convenient method is amplification of the gene from a single-stranded template. The template is generally the product of an automated oligonucleotide synthesis. Amplification primers are derived from the 5' and 3' ends of the template and typically incorporate restriction sites chosen with regard to the cloning site of the vector.

If necessary, translational initiation and termination codons can be engineered into the primer sequences. The sequence encoding the protein may be codon-optimized for expression in the particular host. Thus, for example, if the analogue fusion protein is expressed in bacteria, codons are optimized for bacterial usage. Codon optimization is accomplished by automated synthesis of the entire gene or gene region, ligation of multiple oligonucleotides, mutagenesis of the native sequence, or other techniques known to those in the art.

Within a preferred embodiment, the vector is capable of replication in bacterial cells. Thus, the vector may contain a bacterial origin of replication. Preferred bacterial origins of replication include f1-ori and col E1 ori, especially the ori derived from pUC plasmids. Low copy number vectors (e.g., pPD100) may also be used, especially when the product is deleterious to the host. The plasmids also preferably include at least one selectable marker that is functional in the host. A selectable marker gene confers a phenotype on the host that allows transformed cells to be identified and/or selectively grown. Suitable selectable marker genes for bacterial hosts include the chloroamphenicol resistance gene (Cm^r), ampicillin resistance gene (Amp^r), tetracycline resistance gene (Tc^r), kanamycin resistance gene (Kan^r), and others known in the art. To function in selection, some markers may require a complementary deficiency in the host. The vector may also contain a gene coding for a repressor protein, which is capable of repressing the transcription of a promoter that contains a repressor binding site. Altering the physiological conditions of the cell can depress the promoter. For example, a molecule may be added that competitively binds the repressor, or the temperature of the growth media may be altered. Repressor proteins include, but are not limited to the *E. coli* lacI repressor (responsive to induction by IPTG), the temperature sensitive λ cl857 repressor, and the like.

At minimum, the expression vector should contain a promoter sequence. However, other regulatory sequences may also be included. Such sequences include an enhancer, ribosome binding site, transcription termination signal sequence, secretion signal sequence, origin of replication, selectable marker, and the like. The regulatory sequences are operably linked with one another to allow transcription and subsequent translation. In preferred aspects, the plasmids used herein for expression include a promoter designed for expression of the proteins in bacteria. Suitable promoters, including both

constitutive and inducible promoters, are widely available and are well known in the art. Commonly used promoters for expression in bacteria include promoters from T7, T3, T5, and SP6 phages, and the *trp*, *lpp*, and *lac* operons. Hybrid promoters (see, U.S. Patent No. 4,551,433), such as *tac* and *trc*, may also be used. Examples of plasmids for expression in bacteria include the pET expression vectors pET3a, pET 11a, pET 12a-c, and pET 15b (see U.S. Patent 4,952,496; available from Novagen, Madison, WI). Low copy number vectors (e.g., pPD100) can be used for efficient overproduction of peptides deleterious to the *E. coli* host (Dersch et al., *FEMS Microbiol. Lett.* 123: 19, 1994).

10 Bacterial hosts for the T7 expression vectors may contain chromosomal copies of DNA encoding T7 RNA polymerase operably linked to an inducible promoter (e.g., *lacUV* promoter; see, U.S. Patent No. 4,952,496), such as found in the *E. coli* strains HMS174(DE3)pLysS, BL21(DE3)pLysS, HMS174(DE3) and BL21(DE3). T7 RNA polymerase can also be present on plasmids compatible

15 with the T7 expression vector. The polymerase may be under control of a lambda promoter and repressor (e.g., pGP1-2; Tabor and Richardson, *Proc. Natl. Acad. Sci. USA* 82: 1074, 1985).

In some aspects, the sequence of nucleotides encoding the peptide also encodes a secretion signal, such that the resulting peptide is synthesized as a precursor protein (i.e., proprotein), which is subsequently processed and secreted. The resulting secreted peptide or fusion protein may be recovered from the periplasmic space or the fermentation medium. Sequences of secretion signals suitable for use are widely available and are well known (von Heijne, *J. Mol. Biol.* 184:99-105, 1985).

25 The peptide product is isolated by standard techniques, such as affinity, size exclusion, or ionic exchange chromatography, HPLC and the like. An isolated peptide should preferably show a major band by Coomassie blue stain of SDS-PAGE, which is preferably at least 75%, 80%, 90%, or 95% of the purified peptide, polypeptide, or fusion protein.

30 C. TESTING ANTIMICROBIAL CATIONIC PEPTIDES ANALOGS AND DERIVATIVES

Antimicrobial cationic peptides, and analogs or derivatives thereof, of the present invention are assessed, either alone or in combination with an antimicrobial agent or another analog, for their potential as antibiotic therapeutic agents using a series of assays. Preferably, all peptides are initially

35 assessed *in vitro*, the most promising candidates are selected for further

assessment *in vivo*, and then candidates are selected for pre-clinical studies. The *in vitro* assays include measurement of antibiotic activity, toxicity, solubility, pharmacology, secondary structure, liposome permeabilization and the like. *In vivo* assays include assessment of efficacy in animal models, antigenicity, toxicity, and the like. In general, *in vitro* assays are initially performed, followed by *in vivo* assays.

Generally, cationic peptides are initially tested for (1) antimicrobial activity *in vitro*; (2) *in vitro* toxicity to normal mammalian cells; and (3) *in vivo* toxicity in an animal model. Cationic peptides that have some antimicrobial activity are preferred, although such activity may not be necessary for enhancing the activity of an antibiotic agent. Also, for *in vivo* use, peptides should preferably demonstrate acceptable toxicity profiles, as measured by standard procedures, where lower toxicity is preferred. Additional assays may be performed to demonstrate that the peptide is not immunogenic and to examine antimicrobial activity *in vivo*.

1. *In vitro* assays

Cationic peptides, including indolicidin analogues, are assayed by, for example, an agarose dilution MIC assay, a broth dilution, time-kill assay, or equivalent methods. Antimicrobial activity is measured as inhibition of growth or killing of a microorganism (e.g., bacteria, fungi).

Briefly, a candidate antimicrobial cationic peptide in Mueller Hinton broth supplemented with calcium and magnesium is mixed with molten agarose. Other broths and agars may be used as long as the peptide can freely diffuse through the medium. The agarose is poured into petri dishes or wells, allowed to solidify, and a test strain is applied to the agarose plate. The test strain is chosen, in part, on the intended application of the peptide. Thus, by way of example, if an indolicidin or analog or derivative thereof with activity against *S. aureus* is desired, a *S. aureus* strain is used. It may be desirable to assay the candidate antimicrobial cationic peptide on several strains and/or on clinical isolates of the test species. Plates are incubated overnight and inspected visually for bacterial growth. A minimum inhibitory concentration (MIC) of a cationic peptide is the lowest concentration of peptide that completely inhibits growth of the organism. Peptides that exhibit good activity against the test strain, or group of strains, typically having an MIC of less than or equal to 16 $\mu\text{g/ml}$ are selected for further testing. Preferred antimicrobial

cationic peptides or analogs or derivatives thereof may be microbicidal or microbistatic.

Alternatively, time kill curves can be used to determine the difference in growth (e.g., bacterial colony counts) over a set time period, typically 24 hours. Briefly, a suspension of organisms at a known concentration is prepared and a candidate peptide is added. Aliquots of the suspension are removed at set times, diluted, plated on medium, incubated, and counted. MIC is measured as the lowest concentration of peptide that completely inhibits growth of the organism and, in general, lower MIC values are preferred.

Solubility of the peptide in a solvent, broth, or co-solvent system as described herein is an additional parameter that may be examined. Several different assays may be used, such as appearance in buffer. Briefly, a candidate antimicrobial cationic peptide or analog or derivative thereof may be contacted with a solvent, broth, or co-solvent system, and the appearance evaluated according to a scale that ranges from (a) clear, no precipitate, (b) light, diffuse precipitate, to (c) cloudy, heavy precipitate. In general, less precipitate is more desirable, but some precipitate may be acceptable. To assess the level of solubility, for example, a person having ordinary skill in the art may inspect the combination visually, or a variety of spectrophotometric techniques may be used, such as by U.V. or visible light absorbance at the appropriate wavelength.

Additional *in vitro* assays may be carried out to assess the potential of a candidate peptide or analog or derivative thereof as a therapeutic agent. Such assays include peptide solubility in formulations, pharmacology and stability in blood or plasma, serum protein binding, analysis of secondary structure (e.g., by circular dichroism), liposome permeabilization, and bacterial membrane permeabilization. In general, a preferred embodiment includes a candidate peptide analog or derivative thereof that is soluble, is active in biological fluids, is stable, and has generally greater antimicrobial activity than the natural peptide (e.g., indolicidin).

2. *In vivo* assays

Peptides and analogs or derivative thereof, selected on the basis of the results from the *in vitro* assays can be further tested *in vivo* for efficacy, stability, and the like. A variety of methods and animal models are available to assess the antimicrobial activity of selected candidate peptides and analogs or

derivative thereof *in vivo* for their ability to ameliorate microbial infections. Within these assays, a peptide is useful as a therapeutic if inhibition of microbial growth compared to inhibition with the vehicle alone is statistically significant. This measurement can be made directly from cultures isolated from body fluids or sites, or indirectly by assessing survival rates of infected animals.

For assessment of antibacterial activity of candidate peptide analogs and derivatives as compared to natural peptides, several animal models are available, such as acute infection models including those in which (a) normal mice receive a lethal dose of microorganisms, (b) neutropenic mice receive a lethal dose of microorganisms, or (c) rabbits receive an inoculum of microorganisms in the heart, and chronic infection models. The model selected will depend, in part, on the intended clinical indication of the peptide and/or analog or derivative thereof.

By way of example and not limitation, a normal mouse model is used to inoculate mice, intraperitoneally (i.p.) or intravenously (i.v.), with a lethal dose of bacteria. Typically, the dose is such that 90-100% of animals die within 2 days. The choice of a microorganism strain for this assay depends, in part, upon the intended application of the antimicrobial cationic peptide or analog or derivative thereof, and in the accompanying examples, assays are carried out with three different *Staphylococcus* strains. Briefly, shortly before or after inoculation with the microorganism of choice (generally within 60 minutes), peptides or analogs or derivatives thereof in a suitable formulation buffer (as described herein) is injected. Multiple injections of peptides may be administered. Animals are observed for up to 8 days post-infection, and the survival of animals is recorded. Successful treatment either rescues animals from death or delays death to a statistically significant level, as compared with non-treatment, control animals. Antimicrobial cationic peptide analogs or derivatives thereof that show better efficacy than the natural peptides, such as indolicidins, are preferred.

Furthermore, for *in vivo* use, low immunogenicity is preferred. To measure immunogenicity, peptides are injected into normal animals, generally rabbits. At various times after single or multiple injections, serum is obtained and tested for antibody reactivity to the peptide or analog or derivative thereof. Antibodies to peptides or analogs or derivatives thereof may be identified by ELISA, immunoprecipitation assays, western blots, and other methods known in the art (see *Antibodies: A Laboratory Manual*, 1988). In a preferred

embodiment the antibody of interest has undetectable, or minimally detectable, reactivity with the candidate peptides or analogs or derivative thereof. In addition, pharmacokinetics of the candidate peptides or analogs or derivatives in animals and histopathology of animals treated with the peptides may be
5 determined.

Selection of antimicrobial cationic peptides and analogs or derivatives thereof as potential therapeutics is typically based on *in vitro* and *in vivo* assay results. In general, peptides that exhibit low immunogenicity, good *in vivo* stability, and high efficacy at low dose levels are preferred candidate
10 antimicrobial cationic peptides and analogs or derivatives thereof.

D. CATIONIC PEPTIDE FORMULATIONS AND THERAPEUTIC METHODS

As noted above, the present invention provides methods for treating and preventing infections by administering to a patient a therapeutically effective amount of an antimicrobial cationic peptide, preferably an indolicidin or
15 analog or derivative thereof, as described herein. The antimicrobial cationic peptide is preferably part of a pharmaceutical composition when used in the methods of the present invention. The pharmaceutical composition will include at least one of a pharmaceutically acceptable vehicle, carrier, diluent, or excipient, in addition to one or more antimicrobial cationic peptide and,
20 optionally, other components. Pharmaceutically acceptable excipients for therapeutic use are well known in the pharmaceutical art, and are described herein and, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro, ed., 18th Edition, 1990) and in *CRC Handbook of Food, Drug, and Cosmetic Excipients*, CRC Press LLC (S.C. Smolinski, ed.,
25 1992).

The therapeutic efficacy of an antimicrobial cationic peptide composition according to the present invention is based on a successful clinical outcome and does not require 100% elimination of the microorganisms involved in the infection. Achieving a level of antimicrobial activity at the site of infection
30 that allows the host to survive, resolve the infection, or eradicate the causative agent is sufficient. When host defenses are maximally effective, such as in an otherwise healthy individual, only a minimal antimicrobial effect may suffice. Thus, reducing the organism load by even one log (a factor of 10) may permit the defenses of the host to control the infection. In addition, clinical therapeutic
35 success may depend more on augmenting an early bactericidal effect rather

than on a long-term effect because this allows time for activation of host defense mechanisms. This is especially true for life-threatening infections (e.g., meningitis) and other serious chronic infections (e.g., infective endocarditis).

The formulations of the present invention, having an amount of antimicrobial cationic peptide sufficient to treat or prevent an infection are, for example, particularly suitable for topical (e.g., creams, ointments, skin patches, eye drops, ear drops, shampoos) application or administration. Other typical routes of administration include, without limitation, oral, parenteral, sublingual, bladder wash-out, vaginal, rectal, enteric, suppository, nasal, and inhalation.

5 The term parenteral, as used herein, includes subcutaneous, intravenous, intramuscular, intraarterial, intraabdominal, intraperitoneal, intraarticular, intraocular or retrobulbar, intraaural, intrathecal, intracavitary, intracelial, intraspinal, intrapulmonary or transpulmonary, intrasynovial, and intraurethral injection or infusion techniques. The pharmaceutical compositions of the

10 present invention are formulated so as to allow the antimicrobial cationic peptide contained therein to be bioavailable upon administration of the composition to a subject. The level of peptide in serum and other tissues after administration can be monitored by various well-established techniques, such as bacterial, chromatographic or antibody based (e.g., ELISA) assays. Thus, in

15 certain preferred embodiments, antimicrobial cationic peptides and analogs and derivatives thereof, as described herein, are formulated for topical application to a target site on a subject in need thereof, such as an animal or a human.

The compositions may be administered to a subject as a single dosage unit (e.g., a tablet, capsule, or gel), and the compositions may be

25 administered as a plurality of dosage units (e.g., in aerosol form). For example, the antimicrobial cationic peptide formulations may be sterilized and packaged in single-use, plastic laminated pouches or plastic tubes of dimensions selected to provide for routine, measured dispensing. In one example, the container may have dimensions anticipated to dispense 0.5ml of the antimicrobial cationic

30 peptide composition (e.g., a gel form) to a limited area of the target surface on or in a subject to treat or prevent an infection. A typical target, for example, is in the immediate vicinity of the insertion site of an intravenous catheter, where the target surface usually has an area of about two square centimeters.

The antimicrobial cationic peptide composition may be provided in

35 various forms, depending on the amount and number of different pharmaceutically acceptable excipients present. For example, the peptide

composition may be in the form of a solid, a semi-solid, a liquid, a lotion, a cream, an ointment, a cement, a paste, a gel, or an aerosol. In a preferred embodiment, the peptide formulation is in the form of a gel. The pharmaceutically acceptable excipients suitable for use in the peptide formulation compositions as described herein may include, for example, a viscosity-increasing agent, a buffering agent, a solvent, a humectant, a preservative, a chelating agent, an oleaginous compound, an emollient, an antioxidant, an adjuvant, and the like. The function of each of these excipients is not mutually exclusive within the context of the present invention. For example, glycerin may be used as a solvent or as a humectant or as a viscosity-increasing agent. In one preferred embodiment, the formulation is a composition comprising an antimicrobial cationic peptide, a viscosity-increasing agent, and a solvent, which is useful, for example, at a target site for implanted or indwelling medical devices, as described herein.

The pharmaceutically acceptable excipients noted above are known in the art, yet the unexpected result of the present invention is that particular combinations of excipients afford the antimicrobial cationic peptides and analogs and derivatives thereof stability and prolonged activity when stored at ambient temperature, even in the presence of an alcoholic solvent. Solvents useful in the present compositions are well known in the art and include without limitation water, glycerin, propylene glycol, isopropanol, ethanol, and methanol. In some embodiments, the solvent is glycerin or propylene glycol, preferably at a concentration ranging from about 0.1% to about 20%, more preferably about 5% to about 15%, and most preferably about 9% to 11%. In other embodiments, the solvent is water or ethanol, preferably at a concentration up to about 99%, more preferably up to about 90%, and most preferably up to about 85%. (Unless otherwise indicated, all percentages are on a w/w basis.) In yet other embodiments, the solvent is at least one of water, glycerin, propylene glycol, isopropanol, ethanol, and methanol, preferably is glycerin or propylene glycol and ethanol, more preferably is glycerin and ethanol, and most preferably is glycerin and water. One embodiment is a composition comprising an antimicrobial cationic peptide, a viscosity-increasing agent, a solvent, wherein the solvent comprises at least one of water at a concentration up to 99%, glycerin at a concentration up to 20%, propylene glycol at a concentration up to 20%, ethanol at a concentration up to 99%, and methanol at a concentration up to 99%.

Another useful pharmaceutical excipient of the present invention is a viscosity-increasing agent. In certain embodiments, the antimicrobial cationic peptide compositions of the present invention include a viscosity-increasing agent, including without limitation dextran, polyvinylpyrrolidone, methylcellulose, carboxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose, and combinations thereof. In preferred embodiments, the viscosity-increasing agent is hydroxyethyl cellulose or hydroxypropyl methylcellulose, preferably at a concentration ranging from about 0.5% to about 5%, more preferably from about 1% to about 3%, most preferably from about 1.3% to about 1.7%. In yet other preferred embodiments, the antimicrobial cationic peptide compositions have a first viscosity-increasing agent, such as hydroxyethyl cellulose, hydroxypropyl methylcellulose, dextran, or polyvinylpyrrolidone, and a second viscosity-increasing agent such as hydroxyethyl cellulose, hydroxypropyl methylcellulose, dextran, or polyvinylpyrrolidone. When used as either a first or second viscosity-increasing agent, dextran and polyvinylpyrrolidone are preferably used at a concentration ranging from about 0.1% to about 5% and more preferably from about 0.5% to about 1%. In one preferred embodiment, the first viscosity-increasing agent is hydroxyethyl cellulose at a concentration up to 3% and the second viscosity-increasing agent is hydroxypropyl methylcellulose at a concentration up to 3%. As is known in the art, the amount of viscosity-increasing agent may be increased to shift the form of the composition from a liquid to a gel to a semi-solid form. Thus, the amount of a viscosity-increasing agent used in a formulation may be varied depending on the intended use and location of administration of the peptide compositions provided herein.

In certain applications, it may be desirable to maintain the pH of the antimicrobial cationic peptide composition contemplated by the present invention within a physiologically acceptable range and within a range that optimizes the activity of the peptide or analog or derivative thereof. The antimicrobial cationic peptides of the present invention function best in a composition that is neutral or somewhat acidic, although the peptides will still have antimicrobial and anti-inflammatory activity in a composition that is slightly basic (*i.e.*, pH 8). Accordingly, a composition comprising an antimicrobial cationic peptide, a viscosity-increasing agent, and a solvent, may further comprise a buffering agent. In certain embodiments, the buffering agent comprises a monocarboxylate or a dicarboxylate, and more specifically may be

acetate, fumarate, lactate, malonate, succinate, or tartrate. Preferably, the antimicrobial cationic peptide composition having the buffering agent has a pH ranging from about 3 to about 8, and more preferably from about 3.5 to 7. In another preferred embodiment, the buffering agent is at a concentration ranging
5 from about 1mM to about 200mM, and more preferably from about 2mM to about 20mM, and most preferably about 4mM to about 6mM.

Other optional pharmaceutically acceptable excipients are those that may, for example, aid in the administration of the formulation (*e.g.*, anti-irritant, polymer carrier, adjuvant) or aid in protecting the integrity of the
10 components of the formulation (*e.g.*, anti-oxidants and preservatives. Typically, a 1.0% antimicrobial cationic peptide composition may be stored at 2°C to 8°C. In certain embodiments, the composition comprising an antimicrobial cationic peptide, a viscosity-increasing agent, and a solvent, may further comprise a humectant, preferably sorbitol and the like, or a preservative, preferably benzoic
15 acid, benzyl alcohol, phenoxyethanol, methylparaben, propylparaben, and the like. In certain circumstances, the antimicrobial cationic peptide or analog or derivative thereof may itself function as a preservative of the final therapeutic composition. For example, a preservative is optional in the gel formulations described herein because the gels may be sterilized by autoclaving and,
20 furthermore, show the surprising quality of releasing (*i.e.*, making bioavailable) the antimicrobial cationic peptide at a more optimal rate than other formulations, such as a cream. In addition, particular embodiments may have in a single formulation a humectant, a preservative, and a buffering agent, or combinations thereof. Therefore, a preferred embodiment is a composition comprising an
25 antimicrobial cationic peptide, a viscosity-increasing agent, a solvent, a humectant, and a buffering agent. Another preferred embodiment is a composition comprising an antimicrobial cationic peptide, a viscosity-increasing agent, a buffering agent, and a solvent. In yet another preferred embodiment, the composition comprises an antimicrobial cationic peptide, a buffering agent,
30 and a solvent. Each of the above formulations may be used to treat or prevent infection or to reduce the microflora at a target site, such as a catheter insertion site on a subject (*i.e.*, animal or human).

In yet other embodiments, the composition is in the form of an ointment comprising an antimicrobial cationic peptide (preferably in an amount
35 sufficient to treat or prevent an infection) and an oleaginous compound. For example, oleaginous compound may be petrolatum. In one embodiment, the

oleaginous compound is present at a concentration ranging from about 50% to about 100%, more preferably from about 70% to about 100%, even more preferably from about 80% to about 100%, and most preferably from about 95% to about 100%. In certain other embodiments, the ointment composition may
5 further comprise at least one emollient. The emollients may be present at a concentration ranging from about 1% to about 40%, more preferably from about 5% to about 30%, and more preferably from about 5% to about 10%. In certain preferred embodiments, the emollient may be mineral oil, cetostearyl alcohol, glyceryl stearate, and a combination thereof.

10 In another aspect the composition is in the form of a semi-solid emulsion (e.g., a cream) comprising an antimicrobial cationic peptide (preferably in an amount sufficient to treat or prevent an infection), a solvent, a buffering agent, at least one emollient, and at least one emulsifier. In a preferred embodiment, the semi-solid emulsion or cream further comprises at
15 least one of a humectant (e.g., sorbitol and/or glycerin), an oleaginous compound (e.g., petrolatum), a viscosity increasing agent (e.g., dextran, polyvinylpyrrolidone, hydroxyethyl cellulose, and/or hydroxypropyl methylcellulose), an anti-oxidant (e.g., butylated hydroxytoluene and preferably at a concentration ranging from about 0.01% to about 0.1%), a preservative
20 (e.g., benzoic acid, benzyl alcohol, phenoxyethanol, methylparaben, propylparaben, or a combination thereof), or a combination thereof. In certain preferred embodiments, the emollient may be one or more of stearyl alcohol, cetyl alcohol, and mineral oil. In certain other preferred embodiments, the emulsifiers may be one or more of stearyl alcohol, cetyl alcohol,
25 polyoxyethylene 40 stearate, and glyceryl monostearate. In a preferred embodiment, the emulsifier is present at a concentration ranging from about 1% to about 20%, more preferably from about 5% to about 10, and most preferably from about 1% to about 1.5%. As noted above, the function of each of these emulsifiers and emollients is not mutually exclusive in that an emollient may
30 function as an emulsifier and the emulsifier may function as an emollient, depending on the particular formulation, as is known in the art and is described herein. In certain preferred embodiments the solvent comprises water and the like, and the buffering agent comprises a monocarboxylate or dicarboxylate and the like, as described herein.

35 A subject suitable for treatment with an antimicrobial peptide formulation may be identified by well-established indicators of risk for

developing a disease or well-established hallmarks of an existing disease. For example, indicators of an infection include fever, pus, microorganism positive cultures, inflammation, and the like. Infections that may be treated with antimicrobial cationic peptides provided by the present invention include without
5 limitation those caused by or due to microorganisms, whether the infection is primary, secondary, opportunistic, or the like. Examples of microorganisms include bacteria (e.g., Gram-positive, Gram-negative), fungi, (e.g., yeast and molds), parasites (e.g., protozoans, nematodes, cestodes and trematodes), viruses (e.g., HIV, HSV, VSV), algae, and prions. Specific organisms in these
10 classes are well known (see, for example, Davis *et al.*, *Microbiology*, 3rd edition, Harper & Row, 1980; and Stanier *et al.*, *The Microbial World*, 5th edition, Prentice Hall, 1986). Infections include, but are not limited to, toxic shock syndrome, diphtheria, cholera, typhus, meningitis, whooping cough, botulism, tetanus, pyogenic infections, sinusitis, pneumonia, gingivitis, mucitis, folliculitis,
15 cellulitis, acne and acne vulgaris, impetigo, osteomyelitis, endocarditis, ulcers, burns, dysentery, urinary tract infections, gastroenteritis, anthrax, Lyme disease, syphilis, rubella, septicemia, and plague; as well as primary, secondary, and opportunistic infections associated with, for example, trauma, surgery, endotracheal intubation, tracheostomy, and cystic fibrosis.

20 A subject may have other clinical indications treatable or preventable with the compositions and methods of the present invention, which include without limitation those associated with implantable, indwelling, or similar medical devices, such as intravascular catheters (e.g., intravenous and intra-arterial), right heart flow-directed catheters, Hickman catheters,
25 arteriovenous fistulae, catheters used in hemodialysis and peritoneal dialysis (e.g., silastic, central venous, Tenckhoff, and teflon catheters), vascular access ports, indwelling urinary catheters, urinary catheters, silicone catheters, ventricular catheters, synthetic vascular prostheses (e.g., aortofemoral and femoropopliteal), prosthetic heart valves, prosthetic joints, orthopedic implants,
30 penile implants, shunts (e.g., Scribner, Torkildsen, central nervous system, portasystemic, ventricular, ventriculoperitoneal), intrauterine devices, tampons, contact lenses, dental implants, ureteral stents, pacemakers, implantable defibrillators, tubing, cannulas, probes, blood monitoring devices, needles, and the like. As used herein, "medical device" refers to any device for use in a
35 subject, such as an animal or human.

By way of background, each year over 5 million central venous catheter (CVC) units are sold in the U.S., and it is estimated that 250,000 patients in the U.S. develop bloodstream infections related to CVCs each year. Infections associated with CVCs of various types account for 80-90 % of all vascular catheter-related bloodstream infections (see Maki, D.G., *Infections Caused by Intravascular Devices Used for Infusion Therapy: Pathogenesis, Prevention, and Management*, In: *Infections Associated with Indwelling Medical Devices*, 2nd ed., A.L. Bisno and F.A. Waldvogel (eds.), American Society for Microbiology, Washington, DC, 1994). Prospective studies of short-term, non-cuffed, single or multilumen catheters inserted percutaneously into the subclavian or internal jugular vein have shown that rates of catheter-related septicemia range from 3-5%, with rates of 7-10% in some hospitals (Maki, 1994). Some of the organisms most commonly found to be causing these infections are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecium*, *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Candida albicans*, which account for 95% of reported infections. Therefore, a subject having a catheter or scheduled to have a catheter inserted would be particularly benefited by the antimicrobial cationic peptide compositions and methods of the present invention.

The primary strategies to prevent catheter-related infection are barrier precautions and the use of specialized i.v. teams. Vigorous hand washing and sterile gloves is highly recommended prior to catheter insertion. Other barrier precautions include, for example, a long-sleeved and sterile surgical gown, a mask, a cap, and a large sterile drape. This barrier methodology used in conjunction with specialized i.v. teams has been found to be effective at reducing rates of, for example, catheter-related infection. However, even when these precautions are taken, the longer any type of intravascular device remains in place, the higher the cumulative risk of device-related infection (particularly septicemia). For example, the length of time that non-cuffed CVCs are allowed to remain in place in Intensive Care Unit (ICU) patients is arbitrarily limited to 3 to 7 days in many medical centers. When the continued use of a short-term CVC for more than 4 days is considered essential in an ICU patient, the clinician has three options: (i) leave the catheter in place, accepting that the risk of infection will increase after 4 days; (ii) replace the old catheter over a guidewire at the original insertion site; or (iii) remove the

catheter and place a new catheter at a new site, thereby gaining another 4 days of low risk. None of these options are optimal for the clinician or the patient.

An advantage of the present invention is that antiseptics of a target site for device insertion with an indolicidin or analog or derivative thereof, and formulated as described herein, may be achieved prior to device insertion and/or after device insertion and in combination with barrier precautions, which will reduce the risk of device-associated and other infections. Therefore, the compositions and methods of the present invention are specifically useful, for example, for cutaneous antiseptics to treat or prevent localized infection (e.g., after trauma, surgery, or other medical procedure), and to prevent medical device-related septicemia. Furthermore, as noted above, one benefit of using antimicrobial cationic peptides is the reduction of the risk for selecting antimicrobial-resistant microorganisms.

Uses of antimicrobial cationic peptide formulations of the present invention encompass numerous applications where a topical antimicrobial is useful in the treatment or prevention of infection. For example, burn wound infections remain the most common cause of morbidity and mortality in extensively burned patients. Moreover, infection is the predominant determinant of wound healing, incidence of complications, and outcome of burn patients. The main organisms responsible are *Pseudomonas aeruginosa*, *S. aureus*, *Streptococcus pyogenes*, and various gram-negative organisms. Frequent debridements and establishment of an epidermis or a surrogate, such as a graft or a skin substitute, is essential for prevention of infection. Preferably, the antimicrobial peptide formulations, alone or in combination with antibiotics, is applied to burn wounds as a gel, ointment or cream, and/or administered systemically. Topical application may prevent systemic infection following superficial colonization or eradicate a superficial infection. The antimicrobial peptide composition is preferably administered as a 0.5 to 2% gel, cream, or ointment. Application to the skin could be done once a day or as often as dressings are changed. Systemic administration could be via intravenous, intramuscular or subcutaneous injections or infusions. Other routes of administration known in the art could also be used.

Another use for the present compositions and methods would be in the treatment of surgical wounds, especially those associated with foreign material (e.g., sutures). Nosocomial infections may occur in as many as 71% of all surgical patients, and 40% of those are infections at the operative site.

Despite efforts to prevent infection, it is estimated that between 500,000 and 920,000 surgical wound infections complicate the approximately 23 million surgical procedures performed annually in the United States. The infecting organisms are varied, but *Staphylococci* spp. are important organisms in these infections. Preferably, the antimicrobial peptide formulations, alone or in combination with antibiotics, is applied as an gel, ointment, cream or liquid to the wound site, or as a liquid in the wound prior to and during closure of the wound. Following closure, the antimicrobial peptide composition could also be applied at dressing changes. For surgical or trauma wounds that are infected, the antimicrobial peptide formulation described herein may be applied topically and/or systemically.

Yet another example, sterile gauze dressing has been the standard of care in catheterization for many years, but it has also been demonstrated that transparent polyurethane film dressings are superior because they permit continuous inspection of the catheterization site, they secure the device reliably, and they permit the patients to bathe and shower without saturating the dressing (Maki, 1994). Therefore, certain embodiments of this invention include antimicrobial cationic peptide compositions as described herein for use with sterile gauze or polyurethane film dressings.

Additionally, the compositions and methods of the present invention may be used to reduce the risk of device-related infections by directly coating a medical device prior to insertion at a target site or by impregnating the external surface of a medical device at the time of manufacture. In yet another aspect of this invention, the formulation includes an antimicrobial cationic peptide suitable for impregnating or coating a medical device. Thus, antimicrobial cationic peptides may be formulated as a coating or impregnation material suitable for treating the surfaces of a medical device or its components. In certain embodiments, such coatings and impregnation materials may include covalent and/or non-covalent attachment of an antimicrobial cationic peptide and analog or derivative thereof, to the interior and/or exterior surfaces of a medical device or its components. In other embodiments, such a coating and impregnation material may include the entrapment of an antimicrobial cationic peptide in a hydrogel layer or a bioerodable layer.

Other embodiments include use of antimicrobial cationic peptide coatings, gels, ointments, and impregnation compositions alone or in

conjunction with filter units or catheter components used with, for example, a hemodialysis apparatus. In one embodiment, an arteriovenous shunt, such as a Scribner shunt, may be impregnated, coated, or adapted to a filter containing an antimicrobial cationic peptide. Other embodiments include the same
5 coatings, gels, ointments, and impregnation compositions for use with an arteriovenous fistula. In still another embodiment, a coating may be suitable for use with a woven fiber vascular shunt.

Still other embodiments include use of antimicrobial cationic peptide formulations and methods with temporary access sites used to insert a
10 medical device. In one preferred embodiment, antimicrobial cationic peptide formulations may be used during a femoral vein catheterization. Other preferred embodiments include use of the antimicrobial cationic peptide formulations with catheters, such as vascular dialysis catheters, pulmonary artery catheters, peritoneal dialysis catheters, umbilical catheters, and
15 subclavian vein catheters.

By way of example and not limitation, both local and systemic infection may result from contaminated intravascular devices, such as a CVC, and the organisms typically responsible are coagulase-negative Staphylococci (CoNS), *Staphylococcus aureus*, *Enterococcus* spp, *E. coli* and *Candida* spp.
20 Hence, the antimicrobial cationic peptide or analog or derivative thereof, preferably in the form of a gel or cream, may be applied to the catheter site prior to insertion of the catheter and then again at each dressing change. Preferably, the peptide is at a concentration ranging from about 0.85% to about 1.15%. Therefore, in a typical embodiment, a composition contains an
25 antimicrobial cationic peptide at a concentration ranging from about 0.01% to about 10%; a viscosity-increasing agent selected of dextran, polyvinylpyrrolidone, hydroxyethyl cellulose, or hydroxypropyl methylcellulose; and a solvent of water, glycerin, propylene glycol, isopropanol, ethanol, or methanol; and at a pH ranging from about 3 to about 8.

30 In a preferred embodiment, the present invention is useful in a method for reducing microflora at a target site, comprising applying to the target site a composition comprising an antimicrobial cationic peptide, a viscosity-increasing agent, and a solvent. As used herein, a target site is any site on a subject where there is present, or there is a risk of, a primary or secondary or
35 opportunistic infection (which infection is outside or inside the subject), and is any site where a formulation of the present invention may be administered or

applied. In certain embodiments, the microflora being reduced at the target site may be prokaryotic, eukaryotic, or viral, and preferably is prokaryotic. In other embodiments, the method for reducing microflora at a target site, comprises applying to the target site a composition containing an antimicrobial cationic peptide, a viscosity-increasing agent, and a solvent, and further comprises inserting a medical device at the target site before and/or after applying the composition. In another embodiment, the composition may further contain a buffering agent as described above and may have a pH ranging from about 3.5 to about 7. In addition, the composition may further contain a preservative, such as benzoic acid, benzyl alcohol, phenoxyethanol, methylparaben, propylparaben, and the like. Preferably, the peptide is an indolicidin or analog or derivative thereof, as described herein.

Another preferred embodiment is a composition comprising (a) an antimicrobial cationic peptide wherein the cationic peptide is a peptide of up to 35 amino acids comprising one of the following: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, 11J67CN, 11J68CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN; (b) a viscosity-increasing agent wherein the viscosity-increasing agent is hydroxyethyl cellulose at a concentration of about 1.2% to about 1.8%; (c) a buffer wherein the buffer is lactate at a concentration ranging from about 4mM to about 6mM; (d) a solvent wherein the solvent comprises glycerin at a concentration ranging from about 9% to about 11% and water at a concentration ranging from about 85% to about 90%; and (e) a pH ranging from about 3.5 to about 7. In a more preferred embodiment, the cationic peptide is at a concentration ranging from about 0.8% to about 1.2%. Such compositions would be useful, for example, for topical antisepsis prior to insertion of a device (e.g., catheter) or prosthesis (e.g., synthetic arterial graft). In another embodiment, the composition may be applied to a target site to ameliorate inflammation, such as inflammation associated with an implanted or indwelling medical device.

The compositions and methods of the present invention would be therapeutically effective in treating or preventing acne, including severe acne vulgaris. Acne is due to colonization and infection of hair follicles and sebaceous cysts by *Propionibacterium acne*. Most cases remain mild and do

not lead to scarring, although a subset of patients develop large inflammatory cysts and nodules, which may drain and result in significant scarring. The peptide formulations as described herein may be incorporated into soap, or applied topically as a cream, lotion, or gel to the affected areas. The peptide
5 formulation may be applied either once a day or multiple times during the day, and the length of treatment may be for as long as the lesions are present or to prevent recurrent lesions. Alternatively, the peptide composition may be formulated to be administered orally or systemically to treat or prevent acne lesions. Preferably the peptide composition is formulated for topical
10 administration or application. A preferred embodiment is a composition comprising an antimicrobial cationic peptide, a buffering agent, and a solvent; more preferably a composition, comprising (a) an antimicrobial cationic peptide wherein the cationic peptide is a peptide of up to 35 amino acids comprising one of the following: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN,
15 11F5CN, 11F12CN, 11F17CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, 11J67CN, 11J68CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN; (b) a buffer wherein the buffer is lactate at a concentration ranging from about 4mM to about 6mM; (c) a solvent
20 wherein the solvent comprises ethanol at a concentration ranging from about 45% to about 55% and water at a concentration ranging from about 44% to about 54%; and (d) a pH ranging from about 3.5 to about 7. In a more preferred embodiment, the cationic peptide is at a concentration ranging from about 0.8% to about 1.2%. In another preferred embodiment, the peptide
25 composition may further comprise an acne medicament such as retinoid, vitamin D3, or corticosteroid, and analogs or derivatives thereof. Therefore, in certain preferred methods to reduce microflora, or to treat or prevent an infection, at a target site, the target site may be skin, and the skin may further comprise acne.

30 Another example of the therapeutic value of the compositions and methods of the present invention would be in the treatment of nosocomial infections. For example, infection by *S. aureus* may result in impetiginous lesions or infected wounds, and is associated with increased infection rates following cardiac surgery, hemodialysis, orthopedic surgery and neutropenia,
35 both disease induced and iatrogenic. Nasal and extra-nasal carriage of *Staphylococci* spp. can result in hospital outbreaks of the same *Staphylococci*

strain that is colonizing a patient's or a hospital worker's nasal passage or extra-nasal site. Much attention has been paid to the eradication of nasal colonization, but the results of treatment have been generally unsatisfactory. The use of topical antimicrobial substances, such as bacitracin, tetracycline, and chlorhexidine, results in the suppression of nasal colonization, as opposed to eradication.

Accordingly, a preferred embodiment is a composition comprising an antimicrobial cationic peptide, a viscosity-increasing agent, a solvent, and a preservative; more preferably a composition comprising (a) an antimicrobial cationic peptide wherein the cationic peptide is a peptide of up to 35 amino acids comprising one of the following: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, 11J67CN, 11J68CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN; (b) a viscosity-increasing agent wherein the viscosity-increasing agent is hydroxyethyl cellulose at a concentration of about 1.2% to about 1.8%; (c) a solvent wherein the solvent comprises glycerin at a concentration ranging from about 9% to about 11% and water at a concentration ranging from about 85% to about 90%; (d) a preservative wherein the preservative is benzoic acid at a concentration ranging from about 20mM to about 30mM; and (e) a pH ranging from about 3.5 to about 4.7. In a more preferred embodiment, the cationic peptide is at a concentration ranging from about 0.8% to about 1.2%, or ranging from about 2.5% to about 3.5%.

These preferred compositions may be used in a method for reducing microflora, or for treating or preventing infection, at a target site by applying to the target site the antimicrobial cationic peptide formulations described herein. In another embodiment, the target site may be a mucosa, preferably the mucosa of the nasal passage or anterior naris.

Pharmaceutical compositions of the present invention are administered in a manner appropriate to the infection or disease to be treated. The amount and frequency of administration will be determined by factors such as the condition of the patient, the cause of the infection, and the severity of the infection. Appropriate dosages may be determined by clinical trials, but will generally range from about 0.1 to 50 mg/kg.

In addition, the compositions of the present invention may be used in the manner of common disinfectants or in any situation in which microorganisms are undesirable. For example, these peptides may be used as surface disinfectants, coatings, including covalent bonding, for medical devices, coatings for clothing, such as to inhibit growth of bacteria or repel mosquitoes, in filters for air purification, such as on an airplane, in water purification, constituents of shampoos and soaps, food preservatives, cosmetic preservatives, media preservatives, herbicide or insecticides, constituents of building materials, such as in silicone sealant, and in animal product processing, such as curing of animal hides.

The antimicrobial cationic peptides, particularly the labeled analogs and derivatives thereof, may be used in image analysis and diagnostic assays or for targeting sites in multicellular and single cellular organisms. As a targeting system, the analogues may be coupled with other peptides, proteins, nucleic acids, antibodies, chemical compounds (e.g., fluorescent tags), and the like.

The following Examples are provided by way of illustration and not by way of limitation.

20

EXAMPLES

EXAMPLE 1

SYNTHESIS PURIFICATION AND CHARACTERIZATION OF CATIONIC PEPTIDES AND ANALOGUES

5 Peptide synthesis is based on the standard solid-phase Fmoc protection strategy. The instrument employed is a 9050 Plus PepSynthesiser (PerSeptive BioSystems, Inc.). Polyethylene glycol polystyrene (PEG-PS) graft resins are employed as the solid phase derivatized with an Fmoc-protected amino acid linker for C-terminal amide synthesis. HATU (O-(7-
10 azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) is used as the coupling reagent. During synthesis, coupling steps are continuously monitored to ensure that each amino acid is incorporated in high yield. The peptide is cleaved from the solid-phase resin using trifluoroacetic acid and appropriate scavengers and the crude peptide is purified using
15 preparative reversed-phase chromatography. Typically the peptide is prepared as the trifluoroacetate salt, but other salts, such as acetate, chloride and sulfate, can also be prepared by salt exchange.

 All peptides are analyzed by mass spectrometry to ensure that the product has the expected molecular mass. The product should have a single
20 peak accounting for >95% of the total peak area when subjected to analytical reversed-phase high performance liquid chromatography (RP-HPLC), a separation method that depends on the hydrophobicity of the peptide. In addition, the peptide should show a single band accounting for >90% of the total band intensity when subjected to acid-urea gel electrophoresis, capillary
25 electrophoresis, or any other separation method based on the charge to mass ratio of the peptide.

 Peptide content, the amount of the product that is peptide rather than retained water, salt or solvent, is measured by quantitative amino acid analysis, free amine derivatization or spectrophotometric quantitation. Amino
30 acid analysis also provides information on the ratio of amino acids present in the peptide, which assists in confirming the authenticity of the peptide.

 Peptide analogues and their names are listed below. In this list, and elsewhere, the amino acids are denoted by the one-letter amino acid code and lower case letters represent the D-form of the amino acid.

Table 1. Indolicidin analogs and derivatives thereof and Other Antimicrobial Cationic Peptides

	Apidaecin IA	GNNRPVYIPQPRPPHPRI
5	Deber A2KA2	KKAAAKAAAAAKAAWAAKAAAKKKK
	10	ILPWKWPWWPWRR
	10CN	ILPWKWPWWPWRR
	11	ILKKWPWWPWRRK
	11CN	ILKKWPWWPWRRK
10	11CNR	KRRWPWWPWKKLI
	11A1CN	ILKKFPFFFPRRK
	11A2CN	ILKKIPIPIRRK
	11A3CN	ILKKYPYYPYRRK
	11A4CN	ILKKWPWPWRRK
15	11A5CN	ILKKYPWYPWRRK
	11A6CN	ILKKFPWFPWRRK
	11A7CN	ILKKFPFPWRRK
	11A8CN	ILRYVYYVYRRK
	11A9CN	ILRWPWWPWRRK
20	11A10CN	WWRWPWWPWRRK
	11B1CN	ILRRWPWWPWRRK
	11B2CN	ILRRWPWWPWRRK
	11B3CN	ILKWPWWPWRRK
	11B4CN	ILKKWPWWPWRRK
25	11B5CN	ILKWPWWPWRRK
	11B7CN	ILRWPWWPWRRK
	11B7CNR	KRRWPWWPWRLI
	11B8CN	ILWPWWPWRRK
	11B9CN	ILRRWPWWPWRRR
30	11B10CN	ILKKWPWWPWKKK
	11B16CN	ILRWPWWPWRRKIMILKKAGS
	11B17CN	ILRWPWWPWRRKMILKKAGS
	11B18CN	ILRWPWWPWRRKDMILKKAGS
	11B19CN	ILRWPWRRWPWRRK
35	11B20CN	ILRWPWWPWRRKILMRWPWWPWRRKMAA
	11B32CNR	KRKWPWWPWRLI
	11B36CN	ILKWVWWVWRRK

	11C3CN	ILKKWAWWPWRRK
	11C4CN	ILKKWPWWAWRRK
	11C5CN	WWKKWPWWPWRRK
	11D1CN	LKKWPWWPWRRK
5	11D3CN	PWWPWRRK
	11D4CN	ILKKWPWWPWRRKMILKKAGS
	11D5CN	ILKKWPWWPWRRMILKKAGS
	11D6CN	ILKKWPWWPWRRIMILKKAGS
	11D9M8	WWPWRRK
10	11D10M8	ILKKWPW
	11D11H	ILKKWPWWPWRRKM
	11D12H	ILKKWPWWPWRRM
	11D13H	ILKKWPWWPWRRIM
	11D14CN	ILKKWWWPWRK
15	11D15CN	ILKKWPWWWRK
	11D18CN	WRIWKPKWRLPKW
	11D19CN	CLRWPWWPWRRK
	11E1CN	ILKKWPWWPWRRK
	11E2CN	ILKKWPWWPWRRK
20	11E3CN	ILKKWPWWPWRRK
	11F1CN	ILKKWVWWVWRRK
	11F2CN	ILKKWPWWVWRRK
	11F3CN	ILKKWVWWPWRRK
	11F4CN	ILRWVWWVWRRK
25	11F4CNR	KRRWVWWVWRLI
	11F5CN	ILRRWVWWVWRRK
	11F6CN	ILRWVWWVWWRRK
	11F12CN	RLWVWWVWRRK
	11F17CN	RLWVWWVWRR
30	11F50CN	RLGGGWVWWVWRR
	11F56CN	RLWVWWVWRR
	11F63CN	RLVVWWVWRR
	11F64CN	RLFVWWVFRR
	11F66CN	RLVVWWVWRR
35	11F67CN	rLWVWWVWRR
	11F68CN	RLWVWWVWRR

	11F93CN	WVRLWWRVW
	11G2CN	IKKWPWWPWRK
	11G3CN	ILKKPWWPWRK
	11G4CN	ILKKWWPWRK
5	11G5CN	ILKKWPWWWRK
	11G6CN	ILKKWPWWPRK
	11G7CN	ILKKWPWWPWR
	11G13CN	ILKKWPWWPWK
	11G14CN	ILKKWPWWPWR
10	11G24CN	LWPWWPWRK
	11G25CN	LRWWPWRK
	11G26CN	LRWPWWPW
	11G27CN	WPWWPWRK
	11G28CN	RWWPWRK
15	11H1CN	ALRWPWWPWRK
	11H2CN	IARWPWWPWRK
	11H3CN	ILAWPWWPWRK
	11H4CN	ILRAPWWPWRK
	11H5CN	ILRWAWPWRK
20	11H6CN	ILRWPAWPWRK
	11H7CN	ILRWPAWPWRK
	11H8CN	ILRWPAWWRK
	11H9CN	ILRWPAWARRK
	11H10CN	ILRWPAWPWARK
25	11H11CN	ILRWPAWPWRAK
	11H12CN	ILRWPAWPWRA
	11J01CN	RRIWKPKWRLPKR
	11J02CN	WRWWKPKWRWPKW
	11J02ACN	WRWWKPKWRWPKW
30	11J30CN	WRWWKVAVRWVKW
	11J36CN	WRWWKVWRWVKW
	11J38CN	WRWWKVVRWVKW
	11J58CN	W (Orn) WW (Orn) VAW (Orn) WV (Orn) W
	11J67CN	W (Orn) WW (Orn) P (Orn) W (Orn) WP (Orn) W
35	11J68CN	W (Dab) WW (Dab) P (Dab) W (Dab) WP (Dab) W
	21A1	KKWWRRLSGLKTAGPAIQSVLNK

21A2	KKWWRRALQGLKTAGPAIQSVLNK
21A10	KKWWRRVLKGLSSGPALSNV
22A1	KKWWRRALQALKNGLPALIS
26	KWKSFIAKKLTSAKKVVTTAKPLISS
5 27	KWKLFKKIGIGAVLKVLTTGLPALIS
28	KWKLFKKIGIGAVLKVLTTGLPALKLTK
29	KWKSFIAKKLTAVKKVLTTGLPALIS
29A2	KWKSFIAKNTKVLKKVVTTALPALIS
29A3	KWKSFIAKKLTSAKKVLTTGLPALIS
10 29F1	KWKLFIAKKLTPAVKKVLLTGLPALIS
31	GKPRPYSPIPTSPRIY
REWH 53A5	RLARIVVIRVAR
Nt-Acryloyl-11B7CN	
Nt-glucosyl-11J36CN	
15 Nt-glucosyl-11J38CN	

Nt prefix = N-terminal modification

CN suffix = amidated C-terminus

H suffix = homoserine at C-terminus

20 M suffix = MAP branched peptide

R suffix = retro-synthesized peptide

Orn = ornithine

Dab = diamino butyric acid

Upper case letter = L-enantiomer amino acid

25 Lower case letter = D-enantiomer amino acid

EXAMPLE 2

SYNTHESIS OF MODIFIED PEPTIDES

30 Antimicrobial cationic peptides, such as indolicidin analogs or derivatives thereof, are modified to alter the physical properties of the original peptide, either by use of modified amino acids in synthesis or by post-synthetic modification. Such modifications include: acetylation at the N-terminus, Fmoc-derivatized N-terminus, polymethylation, peracetylation, and branched

derivatives. Peptides modified using the procedures described herein are listed in Table 4.

5 *α -N-terminal acetylation.* Prior to cleaving the peptide from the resin and deprotecting it, the fully protected peptide is treated with N-acetylimidazole in DMF for 1h at room temperature, which results in selective reaction at the α -N-terminus. The peptide is then deprotected/cleaved and purified as for an unmodified peptide.

10 *Fmoc-derivatized α -N-terminus.* If the final Fmoc deprotection step is not carried out, the α -N-terminus Fmoc group remains on the peptide. The peptide is then side-chain deprotected/cleaved and purified as for an unmodified peptide.

15 *Polymethylation.* The purified peptide in a methanol solution is treated with excess sodium bicarbonate, followed by excess methyl iodide. The reaction mixture is stirred overnight at room temperature, extracted with organic solvent, neutralized and purified as for an unmodified peptide. Using this procedure, a peptide is not fully methylated; methylation of 11CN yielded an average of 6 methyl groups. Thus, the modified peptide is a mixture of methylated products.

20 *Caprolactam modification.* A purified peptide in DMF solution is cooled to 0°C on ice with stirring. Added to the peptide solution is 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate and N-methylmorpholine; the reaction mixture is removed from the ice bath and stirred for 1 h, until the reaction mix rises to room temperature. Water is added and the resulting caprolactam peptide solution is purified by C8 RP-HPLC.

25 *Peracetylation.* A purified peptide in DMF solution is treated with N-acetylimidazole for 1h at room temperature. The crude product is concentrated, dissolved in water, lyophilized, re-dissolved in water and purified as for an unmodified peptide. Complete acetylation of primary amine groups is observed.

30 *Four/eight branch derivatives.* The branched peptides are synthesized on a four- or eight-branched core bound to the resin. Synthesis and deprotection/cleavage proceed as for an unmodified peptide. These peptides are purified by dialysis against 4 M guanidine hydrochloride then water, and analyzed by mass spectrometry.

Table 2. Modified Indolicidin analogs and derivatives thereof

Peptide modified	Peptide name	Modification
10	10A	Acetylated α -N-terminus
11	11A	Acetylated α -N-terminus
11CN	11ACN	Acetylated α -N-terminus
11CN	11CNW1	Fmoc-derivatized N-terminus
11CN	11CNX1	Polymethylated derivative
11CN	11CNY1	Peracetylated derivative
11	11M4	Four branch derivative
11	11M8	Eight branch derivative
11B1CN	11B1CNW1	Fmoc-derivatized N-terminus
11B4CN	11B4ACN	Acetylated N-terminus
11B7CN	11B7ACN	Acetylated N-terminus
11B7CN	11B7CNF12	Formylated Lys[12]
11B7	11B7Cap12	Caprolactam Lys[12]
11B9CN	11B9ACN	Acetylated N-terminus
11D9	11D9M8	Eight branch derivative
11D10	11D10M8	Eight branch derivative
11G6CN	11G6ACN	Acetylated α -N-terminus
11G7CN	11G7ACN	Acetylated α -N-terminus

5

EXAMPLE 3**ANTIMICROBIAL ACTIVITY OF CATIONIC PEPTIDES**

A cationic peptide may be tested for antimicrobial activity by using an in vitro assay as described below.

Agarose Dilution Assay

10 The agarose dilution assay measures antimicrobial activity of peptides and peptide analogues. The activity is expressed as the minimum inhibitory concentration (MIC) in $\mu\text{g/ml}$ of the peptide.

In order to mimic *in vivo* conditions, calcium and magnesium supplemented Mueller Hinton broth is used in combination with a low EEO
 15 agarose as the bacterial growth medium. Agarose, rather than agar, is used as

- the charged groups in agar prevent peptide diffusion through the medium. The medium is autoclaved, then cooled to 50-55°C in a water bath before aseptic addition of a peptide solution. The same volume of different concentrations of peptide solution is added to the cooled, molten agarose that is then poured into
- 5 a petri plate to a depth of 3-4 mm and allowed to solidify.

- The bacterial inoculum is adjusted to a 0.5 McFarland turbidity standard (PML Microbiological) and then diluted 1:10 before application on to the agarose plate. The final inoculum applied to the agarose is approximately 10^4 CFU in a 5-8 mm diameter spot. The agarose plates are incubated at 35-
- 10 37°C for 16 to 20 hours.

The MIC is recorded as the lowest concentration of peptide that completely inhibits growth of the organism as determined by visual inspection. Representative MIC values for various peptide analogues against bacteria and yeast are shown in Table 3.

Table 3. Activity of antimicrobial cationic peptides as determined by agarose dilution susceptibility testing

Organism	Strain #	Minimum Inhibitory Concentration ($\mu\text{g/ml}$)						
		11B7CN	11B32CN	11B36CN	11E3CN	11F4CN		
<i>A. calcoaceticus</i>	ACA002	4	2	2	4	4		
<i>E. cloacae</i>	ECL007	128	128	64	>128	64		
<i>E. coli</i>	ECO005	16	16	8	8	4		
<i>K. pneumoniae</i>	KPN001	128	32	8	32	4		
<i>P. aeruginosa</i>	PAE004	128	32	32	64	32		
<i>S. maltophilia</i>	SMA002	16	32	8	64	16		
<i>S. marcescens</i>	SMS003	>128	>128	>128	>128	>128		
<i>E. faecalis</i>	EFS001	64	64	16	64	8		
<i>S. aureus</i>	SAU014	4	8	2	8	4		
<i>S. epidermidis</i>	SEP010	4	2	2	4	2		
<i>S. mitis</i>	SMT014	2	2	2	2	2		
<i>S. pneumoniae</i>	SPN002	4	8	4	4	8		
<i>S. pyogenes</i>	SPY001	1	2	2	1	2		
<i>C. jeikeium</i>	CJK005	0.5	0.5	1	0.5	2		
<i>C. albicans</i>	CAL002	16	16	8	32	32		
<i>C. neoformans</i>	CNE001	4	4	16	4	8		

Table 3. (continued)

Organism	Strain #	Minimum Inhibitory Concentration (µg/ml)					
		11F5CN	11F12CN	11F17CN	11F50CN	11F27CN	11F56CN
<i>A. calcoaceticus</i>	ACA002	4	1	1	2	2	1
<i>E. cloacae</i>	ECL007	128	32	16	>128	128	32
<i>E. coli</i>	ECO005	16	4	2	4	16	2
<i>K. pneumoniae</i>	KPN001	64	8	2	16	32	2
<i>P. aeruginosa</i>	PAE004	64	16	16	>128	32	32
<i>S. maltophilia</i>	SMA002	16	2	1	8	8	2
<i>S. marcescens</i>	SMS003	>128	>128	>128	>128	>128	>128
<i>E. faecalis</i>	EFS001	16	2	2	16	16	2
<i>S. aureus</i>	SAU014	8	2	1	2	8	1
<i>S. epidermidis</i>	SEP010	2	1	1	1	2	1
<i>S. mitis</i>	SMT014	2	1	1	1	4	1
<i>S. pneumoniae</i>	SPN002	4	4	1	2	16	2
<i>S. pyogenes</i>	SPY001	2	0.5	0.5	1	1	1
<i>C. jeikeium</i>	CJK005	0.5	0.5	0.5	0.5	0.5	0.5
<i>C. albicans</i>	CAL002	32	16	32	32	16	16
<i>C. neoformans</i>	CNE001	8	4	4	16	4	8

Table 3. (continued)

Organism	Strain #	Minimum Inhibitory Concentration (µg/ml)						
		11F63CN	11F64CN	11F66CN	11F67CN	11F68CN		
<i>A. calcoaceticus</i>	ACA002	2	2		2	1		
<i>E. cloacae</i>	ECL007	16	16	8	32	32		
<i>E. coli</i>	ECO005	2	2	2	2	2		
<i>K. pneumoniae</i>	KPN001	4	2	2	2	2		
<i>P. aeruginosa</i>	PAE004	8	16	8	16	32		
<i>S. maltophilia</i>	SMA002	0.5	1	2	2	2		
<i>S. marcescens</i>	SMS003	>128	>128	>128	>128	>128		
<i>E. faecalis</i>	EFS001	4	2	2	4	2		
<i>S. aureus</i>	SAU014	2	1	2	2	2		
<i>S. epidermidis</i>	SEP010	1	1	1	1	1		
<i>S. mitis</i>	SMT014	2	1	1	1	1		
<i>S. pneumoniae</i>	SPN002	4	2	2	2	2		
<i>S. pyogenes</i>	SPY001	1	1	1	1	1		
<i>C. jeikeium</i>	CJK005	1	0.5	1	0.5	0.5		
<i>C. albicans</i>	CAL002	16	16	16	32	32		
<i>C. neoformans</i>	CNE001	8	8	8	8	8		

Table 3. (continued)

Organism	Strain #	Minimum Inhibitory Concentration (µg/ml)					
		11F93CN	11G27CN	11J02CN	11J02ACN	11J30CN	11J36CN
<i>A. calcoaceticus</i>	ACA002	4	16	2	2	2	2
<i>E. cloacae</i>	ECL007	64	>128	128	>32	16	32
<i>E. coli</i>	ECO005	4	64	16	16	8	8
<i>K. pneumoniae</i>	KPN001	64	>128	32	32	2	4
<i>P. aeruginosa</i>	PAE004	64	>128	32	64	32	64
<i>S. maltophilia</i>	SMA002	8	64	4	8	4	2
<i>S. marcescens</i>	SMS003	>128	>128	>128	>128	>128	>128
<i>E. faecalis</i>	EFS001	16	>128	64	64	32	16
<i>S. aureus</i>	SAU014	2	16	2	8	2	1
<i>S. epidermidis</i>	SEP010	2	8	2	2	2	1
<i>S. mitis</i>	SMT014	2	8	2	2	1	1
<i>S. pneumoniae</i>	SPN002	4	32	8	16	2	2
<i>S. pyogenes</i>	SPY001	1	2	1	1	2	2
<i>C. jeikeium</i>	CJK005	0.5	0.5	1	0.5	0.5	0.5
<i>C. albicans</i>	CAL002	16	32	16	32	16	16
<i>C. neoformans</i>	CNE001	8	16	4	16	4	4

Table 3. (continued)

Organism	Strain #	Minimum Inhibitory Concentration (µg/ml)				
		11J58CN	11J67CN	11J68CN	Nt- Glucosyl 11J36CN	Nt- Glucosyl 11J38CN
<i>A. calcoaceticus</i>	ACA002	1	1	0.5	1	2
<i>E. cloacae</i>	ECL007	16	32	16	64	32
<i>E. coli</i>	ECO005	2	8	8	4	16
<i>K. pneumoniae</i>	KPN001	2	8	4	2	16
<i>P. aeruginosa</i>	PAE004	16	8	4	16	128
<i>S. maltophilia</i>	SMA002	2	1	4	4	16
<i>S. marcescens</i>	SMS003	>128	>128	128	>128	>128
<i>E. faecalis</i>	EFS001	32	64	16	16	16
<i>S. aureus</i>	SAU014	1	2	1	2	2
<i>S. epidermidis</i>	SEP010	1	1	1	2	2
<i>S. mitis</i>	SMT014	1	0.5	0.5	1	2
<i>S. pneumoniae</i>	SPN002	2	2	1	2	2
<i>S. pyogenes</i>	SPY001	1	<0.25	0.5	1	2
<i>C. jeikeium</i>	CJK005	0.5	<0.25	<0.25	1	1
<i>C. albicans</i>	CAL002	8	8	8	16	16
<i>C. neoformans</i>	CNE001	2	2	1	4	4

EXAMPLE 4

AN IN VITRO DRUG RELEASE METHOD FOR TOPICAL FORMULATIONS
USING LOW-FLOW CELLS

- 5 An *in vitro* drug release method for topical formulations using a low-flow cells is used to examine the release of peptide from experimental formulations. The cell consists of an upper (donor) chamber physically separated from a lower (receptor) chamber by a permeable synthetic membrane (Tuffryn, Gelman). A total of 1 gram of a candidate formulation is placed in the donor chamber. The receptor fluid (distilled water, 37°C) is pumped through the receptor chamber at 2ml/h. Fractions were collected at various hourly intervals. Fractions were collected into vials, and the amount of receptor fluid collected in grams per fraction was recorded. The concentration of drug in the receptor fluid was determined by RP-HPLC on a Nova Pak C8 column. The column was eluted with a gradient from 20% to 40% acetonitrile over 10min. using 0.1% aqueous trifluoroacetic acid, and 0.1% trifluoroacetic acid in acetonitrile, as solvents. The flow rate was 1ml/min.

Table 4. Flow Cell Measurement of *In Vitro* Release

Time Period (h)	Gel 73A	Gel 75A	Gel 76A
0-1	974	789	992
2-3	760	707	777
4-6	722	503	671
7-9	590	414	435
10-12	541	499	455
13-15	396	400	376
16-18	303	300	249
19-21	312	204	220
22-24	309	276	372

The membrane is a 0.45 μm Tuffryn (hydrophilic polysulfone) membrane, the data represents μg of antimicrobial cationic peptide released during the time

interval. The detection limit is 1 µg released per hour. The data in Table 4 shows that gel formulations show excellent initial release that should facilitate rapid antibiotic action upon application. The gel formulations also demonstrate good sustained release of drug. During the 22-24 hour collection, the average concentration of drug released into the receptor fluid ranged from 93 µgrams/ml (Gel 75A) to 124 µgrams/ml (Gel 76A) - well above the MIC values for sensitive organisms.

Table 5. Compositions of Gels and Creams Tested

Ingredient	Gel 73A	Gel 75A	Gel 76A
Antimicrobial Cationic Peptide	1.0	1.0	1.0
Hydroxyethyl Cellulose, NF	1.5	1.5	1.5
Glycerin, USP	10.0	10.0	10.0
Polyvinylpyrrolidone 90	---	1.0	---
0.1M Lactate buffer, pH 4	5.0*	5.0	5.0
Dextran (40,000), USP	---	---	1.0
Purified Water, USP	100.0	100.0	100.0

All entries are grams added/100 grams of gel.

EXAMPLE 5

15 ANTIMICROBIAL ACTIVITY OF AN AQUEOUS 1.0% CATIONIC PEPTIDE GEL

The objective of this study was to assess the antimicrobial activity of 1.0% antimicrobial cationic peptide gel against *Pseudomonas aeruginosa* PA004; *Candida albicans* CA002; *Staphylococcus aureus* SA016; and *Staphylococcus epidermidis* SE010. Briefly, a 2 g portion of 11B7CN 1.0% Gel was aseptically transferred into each of sixteen 50 mL tubes. Four tubes, one for each bacterium, were labeled as day 0, day 1, day 3, and day 7. As a control, a 2 g portion of vehicle (gel without antimicrobial cationic peptide 1.0%) was aseptically transferred into each of sixteen 50 mL tubes and labeled as described above.

25 An inoculum of 1×10^8 CFU/ml for each of the above listed organisms was prepared. For each of the series of tubes designated for each

specific organism, 10 µl of undiluted inoculum was added to the gel in each of the tubes giving a final bacterial concentration of 5×10^5 CFU/g of gel. The contents of the tube were mixed using the handle of a sterile swab.

- The tubes labeled days 1, 3, and 7 were held at ambient
- 5 temperatures and sampled at the indicated time point. The day 0 tubes were sampled immediately. At the time of sampling, 1 g of gel from the test or control tube was removed and streaked on appropriate culture media. Growth of the appropriate organism was observed after 24 and 48 hours of incubation. The remaining 1 g of test or control gel was added to saline and serially diluted.
- 10 Aliquots of 0.1 ml were plated in duplicate on appropriate medium and counted after 24 and 48 hours of incubation.

- The results of this study are described in Table 6. The 1.0% antimicrobial cationic peptide gel has activity against *P. aeruginosa* PA004; *C. albicans* CA002; *S. aureus* SA016; and *S. epidermidis* SE010. Each of the
- 15 organisms was killed immediately upon exposure to the 1.0% antimicrobial cationic peptide gel.

Table 6. Summary of Colony Counts for Four Organisms with 1.0% Antimicrobial Cationic Peptide Gel

Organism Tested: <i>C. albicans</i> (CA002) MIC = 64 ug/ml											
Dilution	Formulation without 11B7CN (48 hour counts)					11B7CN in Formulation (48 hour counts)					
	Time 0	1 Day	3 Days	7 Days		Time 0	1 Day	3 Days	7 Days		
	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)		Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)		
Neat	TNTC*	TNTC	TNTC	TNTC		0	0	0	0		0
10 ⁻¹	1.5 x 10 ⁵	TNTC	TNTC	TNTC		0	0	0	0		0
10 ⁻²	1.2 x 10 ⁵	3.3 x 10 ⁵	5.0 x 10 ⁵	4.1 x 10 ⁵		0	0	0	0		0
10 ⁻³	2.5 x 10 ⁵	3.5 x 10 ⁵	5.0 x 10 ⁵	7.5 x 10 ⁵		0	0	0	0		0
10 ⁻⁴	0	0	3.0 x 10 ⁶	1.5 x 10 ⁶		0	0	0	0		0

* TNTC = too numerous to count

Table 6. (continued)

Organism Tested: <i>P. aeruginosa</i> (PA004) MIC = 128 ug/ml										
	Formulation without Antimicrobial cationic peptide (48 h counts)					Antimicrobial cationic peptide in Formulation (48 h counts)				
	Time 0	1 Day	3 Days	7 Days		Time 0	1 Day	3 Days	7 Days	
Dilution	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)		Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	
Neat	5.3×10^3	1.0×10^2	0	0		0	0	0	0	
10^{-1}	5.5×10^3	0	0	0		0	0	0	0	
10^{-2}	0	0	0	0		0	0	0	0	
10^{-3}	0	0	0	0		0	0	0	0	

Table 6. (continued)

Organism Tested: <i>S. aureus</i> (SA016) MIC = 2 ug/ml										
Formulation without Antimicrobial cationic peptide (48 h counts)					Antimicrobial cationic peptide in Formulation (48 h counts)					
	Time 0	1 Day	3 Days	7 Days ^a	Time 0	1 Day	3 Days	7 Days		
Dilution	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)		
Neat	TNTC*	TNTC	TNTC	TNTC	0	0	0	0		
10 ⁻¹	TNTC	TNTC	TNTC	TNTC	0	0	0	0		
10 ⁻²	4.8 x 10 ⁵	8.4 x 10 ⁵	7.6 x 10 ⁵	4.2 x 10 ⁵	0	0	0	0		
10 ⁻³	3.5 x 10 ⁵	1.0 x 10 ⁶	1.3 x 10 ⁶	6.0 x 10 ⁵	0	0	0	0		

* TNTC = too numerous to count

Table 6. (continued)

Organism Tested: <i>S. epidermidis</i> (SE010) MIC = 4 ug/ml									
	Formulation without Antimicrobial cationic peptide (48 h counts)					Antimicrobial cationic peptide in Formulation (48 h counts)			
	Time 0	1 Day	3 Days	7 Days ^a	Time 0	1 Day	3 Days	7 Days	
Dilution	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	Avg. Colony Count (CFU/g)	
Neat	TNTC*	TNTC	2.1 x 10 ⁴	1.2 x 10 ³	0	0	0	0	
10 ⁻¹	8.2 x 10 ⁴	5.5 x 10 ⁴	1.9 x 10 ⁴	3.0 x 10 ³	0	0	0	0	
10 ⁻²	1.3 x 10 ⁵	4.5 x 10 ⁴	1.0 x 10 ⁴	0	0	0	0	0	
10 ⁻³	1.0 x 10 ⁵	5.0 x 10 ⁴	0	0	0	0	0	0	

* TNTC = too numerous to count

All of the above U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference, in their entirety.

- 5 It will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without departing from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

We claim:

1. A composition, comprising an antimicrobial cationic peptide, a viscosity-increasing agent, and a solvent.
2. The composition according to claim 1, further comprising a buffering agent.
3. The composition according to claim 2 wherein the buffering agent comprises a monocarboxylate or a dicarboxylate.
4. The composition according to claim 2 wherein the buffering agent is selected from the group consisting of acetate, fumarate, lactate, malonate, succinate, and tartrate.
5. The composition according to claim 2 having a pH ranging from about 3 to about 8.
6. The composition according to claim 2 comprising the buffering agent at a concentration ranging from about 1mM to about 200mM.
7. The composition according to claim 1, further comprising a humectant.
8. The composition according to claim 7 wherein the humectant is sorbitol or glycerol.
9. The composition according to claim 1 wherein the solvent is selected from the group consisting of water, glycerin, propylene glycol, isopropanol, ethanol, and methanol.
10. The composition according to claim 1 wherein the solvent is glycerin.

11. The composition according to claim 10 comprising glycerin at a concentration ranging from about 0.1% to about 20%.
12. The composition according to claim 10 comprising glycerin at a concentration ranging from about 9% to about 11%.
13. The composition according to claim 1 wherein the solvent is propylene glycol.
14. The composition according to claim 13 comprising propylene glycol at a concentration ranging from about 0.1% to about 20%.
15. The composition according to claim 13 comprising propylene glycol at a concentration ranging from about 9% to about 11%.
16. The composition according to claim 1 wherein the solvent is water.
17. The composition according to claim 1 wherein the solvent is ethanol.
18. The composition according to any one of claims 1, 2, or 7, further comprising a preservative.
19. The composition according to claim 18 wherein the preservative comprises benzoic acid, benzyl alcohol, phenoxyethanol, methylparaben, propylparaben, or a combination thereof.
20. The composition according to claim 1 wherein the viscosity-increasing agent is selected from the group consisting of dextran, polyvinylpyrrolidone, hydroxyethyl cellulose, and hydroxypropyl methylcellulose.
21. The composition according to claim 1 wherein the viscosity-increasing agent is hydroxyethyl cellulose.

22. The composition according to claim 21 comprising hydroxyethyl cellulose at a concentration ranging from about 0.5% to about 5%.

23. The composition according to claim 21 comprising hydroxyethyl cellulose at a concentration ranging from about 1% to about 3%.

24. The composition according to claim 1 wherein the viscosity-increasing agent is hydroxypropyl methylcellulose.

25. The composition according to claim 24 comprising hydroxypropyl methylcellulose at a concentration ranging from about 1% to about 3%.

26. The composition according to claim 21, further comprising a second viscosity-increasing agent selected from the group consisting of dextran, polyvinylpyrrolidone, and hydroxypropyl methylcellulose.

27. The composition according to claim 26 wherein the second viscosity-increasing agent is dextran.

28. The composition according to claim 27 comprising dextran at a concentration ranging from about 0.1% to about 5%.

29. The composition according to claim 27 comprising dextran at a concentration ranging from about 0.5% to about 1%.

30. The composition according to claim 26 wherein the second viscosity-increasing agent is polyvinylpyrrolidone.

31. The composition according to claim 30 comprising polyvinylpyrrolidone at a concentration ranging from about 0.1% to about 5%.

32. The composition according to claim 30 comprising polyvinylpyrrolidone at a concentration ranging from about 0.5% to about 1%.

33. The composition according to claim 26 wherein the second viscosity-increasing agent is hydroxypropyl methylcellulose.

34. The composition according to claim 33 comprising hydroxypropyl methylcellulose at a concentration ranging from about 1% to about 3%.

35. The composition according to claim 24, further comprising a second viscosity-increasing agent of dextran or polyvinylpyrrolidone.

36. The composition according to claim 35 wherein the second viscosity-increasing agent is dextran.

37. The composition according to claim 36 comprising dextran at a concentration ranging from about 0.1% to about 5%.

38. The composition according to claim 36 comprising dextran at a concentration ranging from about 0.5% to about 1%.

39. The composition according to claim 35 wherein the second viscosity-increasing agent is polyvinylpyrrolidone.

40. The composition according to claim 39 comprising polyvinylpyrrolidone at a concentration ranging from about 0.1% to about 5%.

41. The composition according to claim 39 comprising polyvinylpyrrolidone at a concentration ranging from about 0.5% to about 1%.

42. The composition according to claim 1 wherein the solvent comprises at least one of water, glycerin, propylene glycol, isopropanol, ethanol, and methanol.

43. The composition according to claim 42 wherein the solvent comprises at least one of water at a concentration up to 99%, glycerin at a concentration up to 20%, propylene glycol at a concentration up to 20%,

ethanol at a concentration up to 99%, and methanol at a concentration up to 99%.

44. The composition according to claim 1 wherein the cationic peptide is an indolicidin or an analog or derivative thereof.

45. The composition according to claim 1 comprising cationic peptide at a concentration ranging from about 0.01% to about 10%.

46. The composition according to claim 1 comprising cationic peptide at a concentration ranging from about 0.5% to about 1.5%.

47. The composition according to any one of claims 1-46 wherein the cationic peptide is a peptide of up to 35 amino acids, comprising one of the following sequences: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F27CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN.

48. The composition according to any one of claims 1-46 wherein the cationic peptide comprises the amino acid sequence 11B7CN.

49. The composition according to any one of claims 1-46 wherein the cationic peptide comprises the amino acid sequence 11F4CN.

50. The composition according to any one of claims 1-46 wherein the cationic peptide comprises the amino acid sequence 11J02CN.

51. The composition according to any one of claims 1-46 wherein the cationic peptide comprises the amino acid sequence 11J30CN.

52. The composition according to any one of claims 1-46 wherein the cationic peptide comprises the amino acid sequence 11J36CN.

53. The composition according to any one of claims 1-46 wherein the cationic peptide comprises the amino acid sequence Nt-acryloyl-11B7CN.

54. The composition according to any one of claims 1-46 wherein the cationic peptide comprises the amino acid sequence Nt-glucosyl-11J36CN.

55. A composition, comprising an antimicrobial cationic peptide, a viscosity-increasing agent, a solvent, a humectant, and a buffering agent.

56. The composition according to claim 55 wherein the buffering agent comprises a monocarboxylate or a dicarboxylate.

57. The composition according to claim 55 wherein the buffering agent is selected from the group consisting of acetate, fumarate, lactate, malonate, succinate, and tartrate.

58. The composition according to claim 55 having a pH ranging from about 3 to 8.

59. The composition according to claim 55 comprising the buffering agent at a concentration ranging from about 1mM to about 200mM.

60. The composition according to claim 55 wherein the humectant is sorbitol or glycerol.

61. The composition according to claim 55 wherein the solvent is selected from the group consisting of water, glycerin, propylene glycol, ethanol, and methanol.

62. The composition according to claim 55 wherein the solvent is glycerin.

63. The composition according to claim 62 comprising glycerin at a concentration ranging from about 0.1% to about 20%.

64. The composition according to claim 62 comprising glycerin at a concentration ranging from about 9% to about 11%.

65. The composition according to claim 55 wherein the solvent is propylene glycol.

66. The composition according to claim 65 comprising propylene glycol at a concentration ranging from about 0.1% to about 20%.

67. The composition according to claim 65 comprising propylene glycol at a concentration ranging from about 9% to about 11%.

68. The composition according to claim 55 wherein the solvent is water.

69. The composition according to claim 55 wherein the solvent is ethanol.

70. The composition according to claim 55, further comprising a preservative.

71. The composition according to claim 70 wherein the preservative comprises benzoic acid, benzyl alcohol, phenoxyethanol, methylparaben, propylparaben, or a combination thereof.

72. The composition according to claim 55 wherein the viscosity-increasing agent is selected from the group consisting of dextran, polyvinylpyrrolidone, hydroxyethyl cellulose, and hydroxypropyl methylcellulose.

73. The composition according to claim 55 wherein the viscosity-increasing agent is hydroxyethyl cellulose.

74. The composition according to claim 73 comprising hydroxyethyl cellulose at a concentration ranging from about 0.5% to about 5%.

75. The composition according to claim 73 comprising hydroxyethyl cellulose at a concentration ranging from about 1% to about 3%.

76. The composition according to claim 55 wherein the viscosity-increasing agent is hydroxypropyl methylcellulose.

77. The composition according to claim 76 comprising hydroxypropyl methylcellulose at a concentration ranging from about 0.5% to about 5%.

78. The composition according to claim 76 comprising hydroxypropyl methylcellulose at a concentration ranging from about 1% to about 3%.

79. The composition according to claim 73, further comprising a second viscosity-increasing agent selected from the group consisting of dextran, polyvinylpyrrolidone, and hydroxypropyl methylcellulose.

80. The composition according to claim 79 wherein the second viscosity-increasing agent is dextran.

81. The composition according to claim 80 comprising dextran at a concentration ranging from about 0.1% to about 5%.

82. The composition according to claim 80 comprising dextran at a concentration ranging from about 0.5% to about 1%.

83. The composition according to claim 79 wherein the second viscosity-increasing agent is polyvinylpyrrolidone.

84. The composition according to claim 83 comprising polyvinylpyrrolidone at a concentration ranging from about 0.1% to about 5%.

85. The composition according to claim 83 comprising polyvinylpyrrolidone at a concentration ranging from about 0.5% to about 1%.

86. The composition according to claim 76, further comprising a second viscosity-increasing agent of dextran or polyvinylpyrrolidone.

87. The composition according to claim 86 wherein the second viscosity-increasing agent is dextran.

88. The composition according to claim 87 comprising dextran at a concentration ranging from about 0.1% to about 5%.

89. The composition according to claim 87 comprising dextran at a concentration ranging from about 0.5% to about 1%.

90. The composition according to claim 86 wherein the second viscosity-increasing agent is polyvinylpyrrolidone.

91. The composition according to claim 90 comprising polyvinylpyrrolidone at a concentration ranging from about 0.1% to about 5%.

92. The composition according to claim 90 comprising polyvinylpyrrolidone at a concentration ranging from about 0.5% to about 1%.

93. The composition according to claim 55 wherein the solvent comprises at least one of water, glycerin, propylene glycol, ethanol, and methanol.

94. The composition according to claim 93 wherein the solvent comprises at least one of water at a concentration up to 99%, glycerin at a concentration up to 20%, propylene glycol at a concentration up to 20%, ethanol at a concentration up to 99%, and methanol at a concentration up to 99%.

95. The composition according to claim 55 wherein the cationic peptide is indolicidin or an analog or derivative thereof.

96. The composition according to claim 55 comprising cationic peptide at a concentration ranging from about 0.01% to about 10%.

97. The composition according to claim 55 comprising cationic peptide at a concentration ranging from about 0.5% to about 1.5%.

98. The composition according to any one of claims 55-97 wherein the cationic peptide is a peptide of up to 35 amino acids, comprising one of the following sequences: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F27CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN.

99. The composition according to any one of claims 55-97 wherein the cationic peptide comprises the amino acid sequence 11B7CN.

100. The composition according to any one of claims 55-97 wherein the cationic peptide comprises the amino acid sequence 11F4CN.

101. The composition according to any one of claims 55-97 wherein the cationic peptide comprises the amino acid sequence 11J02CN.

102. The composition according to any one of claims 55-97 wherein the cationic peptide comprises the amino acid sequence 11J30CN.

103. The composition according to any one of claims 55-97 wherein the cationic peptide comprises the amino acid sequence 11J36CN.

104. The composition according to any one of claims 55-97 wherein the cationic peptide comprises the amino acid sequence Nt-acryloyl-11B7CN.

105. The composition according to any one of claims 55-97 wherein the cationic peptide comprises the amino acid sequence Nt-glucosyl-11J36CN.

106. A composition, comprising an antimicrobial cationic peptide, a buffering agent, and a solvent.

107. The composition according to claim 106 wherein the buffering agent comprises a monocarboxylate or a dicarboxylate.

108. The composition according to claim 106 wherein the buffering agent is selected from the group consisting of acetate, fumarate, lactate, malonate, succinate, and tartrate.

109. The composition according to claim 106 having a pH ranging from about 3 to 8.

110. The composition according to claim 106 comprising the buffering agent at a concentration ranging from about 1mM to about 200mM.

111. The composition according to claim 106 comprising the buffering agent at a concentration ranging from about 4mM to about 6mM.

112. The composition according to claim 106, further comprising a humectant.

113. The composition according to claim 112 wherein the humectant is sorbitol or glycerol.

114. The composition according to claim 106 wherein the solvent is selected from the group consisting of water, glycerin, propylene glycol, ethanol, isopropanol, and methanol.

115. The composition according to claim 106 wherein the solvent is glycerin.

116. The composition according to claim 115 comprising glycerin at a concentration ranging from about 9% to about 11%.

117. The composition according to claim 106 wherein the solvent is propylene glycol.

118. The composition according to claim 117 comprising propylene glycol at a concentration ranging from about 9% to about 11%.

119. The composition according to claim 106 wherein the solvent is water.

120. The composition according to claim 106 wherein the solvent is ethanol.

121. The composition according to claim 106 wherein the solvent is isopropanol.

122. The composition according to claim 106 wherein the solvent is methanol.

123. The composition according to any one of claims 106 or 112, further comprising a preservative.

124. The composition according to claim 123 wherein the preservative comprises benzoic acid, benzyl alcohol, phenoxyethanol, methylparaben, propylparaben, or a combination thereof.

125. The composition according to claim 106, further comprising at least one viscosity-increasing agent selected from the group consisting of dextran, polyvinylpyrrolidone, hydroxyethyl cellulose, and hydroxypropyl methylcellulose.

126. The composition according to claim 125 wherein the viscosity-increasing agent is hydroxyethyl cellulose.

127. The composition according to claim 126 comprising hydroxyethyl cellulose at a concentration ranging from about 1% to about 3%.

128. The composition according to claim 125 wherein the viscosity-increasing agent is hydroxypropyl methylcellulose.

129. The composition according to claim 128 comprising hydroxypropyl methylcellulose at a concentration ranging from about 1% to about 3%.

130. The composition according to claim 126, further comprising a second viscosity-increasing agent, wherein the second viscosity-increasing agent is hydroxypropyl methylcellulose.

131. The composition according to claim 130 comprising hydroxyethyl cellulose at a concentration up to about 3% and hydroxypropyl methylcellulose at a concentration up to about 3%.

132. The composition according to claim 106 wherein the solvent comprises at least one of water, glycerin, propylene glycol, isopropanol, ethanol, and methanol.

133. The composition according to claim 132 wherein the solvent comprises at least one of water at a concentration up to 99%, glycerin at a concentration up to 20%, propylene glycol at a concentration up to 20%, isopropanol at a concentration up to 99%, ethanol at a concentration up to 99%, and methanol at a concentration up to 99%.

134. The composition according to claim 106 wherein the cationic peptide is indolicidin or an analog or derivative thereof.

135. The composition according to claim 106 comprising cationic peptide at a concentration ranging from about 0.01% to 10%.

136. The composition according to claim 106 comprising cationic peptide at a concentration ranging from about 0.5% to 1.5%.

137. The composition according to any one of claims 106-136 wherein the cationic peptide is a peptide of up to 35 amino acids, comprising

one of the following sequences: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F27CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN.

138. The composition according to any one of claims 106-136 wherein the cationic peptide comprises the amino acid sequence 11B7CN.

139. The composition according to any one of claims 106-136 wherein the cationic peptide comprises the amino acid sequence 11F4CN.

140. The composition according to any one of claims 106-136 wherein the cationic peptide comprises the amino acid sequence 11J02CN.

141. The composition according to any one of claims 106-136 wherein the cationic peptide comprises the amino acid sequence 11J30CN.

142. The composition according to any one of claims 106-136 wherein the cationic peptide comprises the amino acid sequence 11J36CN.

143. The composition according to any one of claims 106-136 wherein the cationic peptide comprises the amino acid sequence Nt-acryloyl-11B7CN.

144. The composition according to any one of claims 106-136 wherein the cationic peptide comprises the amino acid sequence Nt-glucosyl-11J36CN.

145. The composition according to claim 106, further comprising an acne medicament selected from the group consisting of retinoid, vitamin D3, and corticosteroid, and analogs or derivatives thereof.

146. A composition, comprising an antimicrobial cationic peptide at a concentration ranging from about 0.01% to about 10%; a viscosity-increasing agent selected from the group consisting of dextran,

polyvinylpyrrolidone, hydroxyethyl cellulose, and hydroxypropyl methylcellulose; and a solvent selected from the group consisting of water, glycerin, propylene glycol, isopropanol, ethanol, and methanol; at a pH ranging from about 3 to about 8.

147. The composition according to claim 146, further comprising a buffering agent.

148. The composition according to claim 147 having a pH ranging from about 3.5 to about 7.

149. The composition according to claim 147 wherein the buffering agent is selected from the group consisting of acetate, fumarate, lactate, malonate, succinate, and tartrate.

150. The composition according to claim 147 wherein the buffering agent comprises a monocarboxylate or a dicarboxylate.

151. The composition according to claim 147 comprising the buffering agent at a concentration ranging from about 1mM to about 200mM.

152. The composition according to claim 147 comprising the buffering agent at a concentration ranging from about 4mM to about 6mM.

153. The composition according to claim 146 wherein the viscosity-increasing agent is hydroxyethyl cellulose.

154. The composition according to claim 153 comprising hydroxyethyl cellulose at a concentration ranging from about 1% to about 2%.

155. The composition according to claim 146 wherein the solvent is glycerin.

156. The composition according to claim 146 comprising glycerin at a concentration ranging from about 9% to about 11%.

157. The composition according to claim 146 wherein the solvent is water.

158. The composition according to claim 146 wherein the solvent is ethanol.

159. The composition according to claim 146, further comprising a preservative.

160. The composition according to claim 159 wherein the preservative comprises benzoic acid, benzyl alcohol, phenoxyethanol, methylparaben, propylparaben, or a combination thereof.

161. The composition according to claim 146 wherein the cationic peptide is indolicidin or an analog or derivative thereof.

162. The composition according to any one of claims 146-161 wherein the cationic peptide is a peptide of up to 35 amino acids, comprising one of the following sequences: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F27CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN.

163. The composition according to any one of claims 146-161 wherein the cationic peptide comprises the amino acid sequence 11B7CN.

164. The composition according to any one of claims 146-161 wherein the cationic peptide comprises the amino acid sequence 11F4CN.

165. The composition according to any one of claims 146-161 wherein the cationic peptide comprises the amino acid sequence 11J02CN.

166. The composition according to any one of claims 146-161 wherein the cationic peptide comprises the amino acid sequence 11J30CN.

167. The composition according to any one of claims 146-161 wherein the cationic peptide comprises the amino acid sequence 11J36CN.

168. The composition according to any one of claims 146-161 wherein the cationic peptide comprises the amino acid sequence Nt-acryloyl-11B7CN.

169. The composition according to any one of claims 146-161 wherein the cationic peptide comprises the amino acid sequence Nt-glucosyl-11J36CN.

170. A composition, comprising:

a) an antimicrobial cationic peptide wherein the cationic peptide is a peptide of up to 35 amino acids, comprising one of the following sequences: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F27CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN;

b) a viscosity-increasing agent wherein the viscosity-increasing agent is hydroxyethyl cellulose at a concentration of about 1.2% to about 1.8%;

c) a buffer wherein the buffer is lactate at a concentration ranging from about 4mM to about 6mM;

d) a solvent wherein the solvent comprises glycerin at a concentration ranging from about 9% to about 11% and water at a concentration ranging from about 85% to about 90%; and

e) a pH ranging from about 3.5 to about 7.

171. A composition, comprising:

a) an antimicrobial cationic peptide wherein the cationic peptide is a peptide of up to 35 amino acids, comprising one of the following sequences: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F27CN, 11F50CN, 11F56CN, 11F63CN,

11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN;

b) a buffer wherein the buffer is lactate at a concentration ranging from about 4mM to about 6mM;

c) a solvent wherein the solvent comprises ethanol at a concentration ranging from about 45% to about 55% and water at a concentration ranging from about 44% to about 54%; and

d) a pH ranging from about 3.5 to about 7.

172. A composition, comprising:

a) an antimicrobial cationic peptide wherein the cationic peptide is a peptide of up to 35 amino acids, comprising one of the following sequences: 11B7CN, 11B32CN, 11B36CN, 11E3CN, 11F4CN, 11F5CN, 11F12CN, 11F17CN, 11F27CN, 11F50CN, 11F56CN, 11F63CN, 11F64CN, 11F66CN, 11F67CN, 11F68CN, 11F93CN, 11G27CN, 11J02CN, 11J02ACN, 11J30CN, 11J36CN, 11J58CN, Nt-acryloyl-11B7CN, Nt-glucosyl-11J36CN, or Nt-glucosyl-11J38CN;

b) a viscosity-increasing agent wherein the viscosity-increasing agent is hydroxyethyl cellulose at a concentration of about 1.2% to about 1.8%;

c) a solvent wherein the solvent comprises glycerin at a concentration ranging from about 9% to about 11% and water at a concentration ranging from about 85% to about 90%;

d) a preservative wherein the preservative is benzoic acid at a concentration ranging from about 20mM to about 30mM; and

e) a pH ranging from about 3.5 to about 4.7.

173. A composition according to claims 170-172 wherein the cationic peptide is at a concentration ranging from about 0.8% to about 1.2%.

174. A composition according to claim 172 wherein the cationic peptide is at a concentration ranging from about 2.5% to about 3.5%.

175. A method for reducing microflora at a target site, comprising applying to the target site a composition comprising an antimicrobial cationic peptide, a viscosity-increasing agent, and a solvent.

176. The method of claim 175 wherein the microflora is a prokaryotic organism, a eukaryotic organism, or a virus.

177. The method of claim 175, further comprising inserting a medical device at the target site after applying the composition.

178. The method of claim 175, further comprising inserting a medical device at the target site before applying the composition.

179. The method according to any one of claims 177 or 178 wherein the device comprises a catheter.

180. The method according to any one of claims 177 or 178, further comprising applying the composition to the device prior to inserting the device at the target site.

181. The method according to claim 179 wherein the catheter comprises a central venous catheter.

182. The method according to claim 179 wherein the catheter is selected from the group consisting of a vascular dialysis catheter, a pulmonary artery catheter, a peritoneal dialysis catheter, and an umbilical catheter.

183. The method of claim 175 wherein the target site is a skin.

184. The method of claim 183 wherein the skin further comprises acne.

185. The method of claim 175 wherein the target site is a mucosa.

186. The method of claim 185 wherein the mucosa further comprises a nasal passage.

187. The method of claim 186 wherein the nasal passage is an anterior naris.

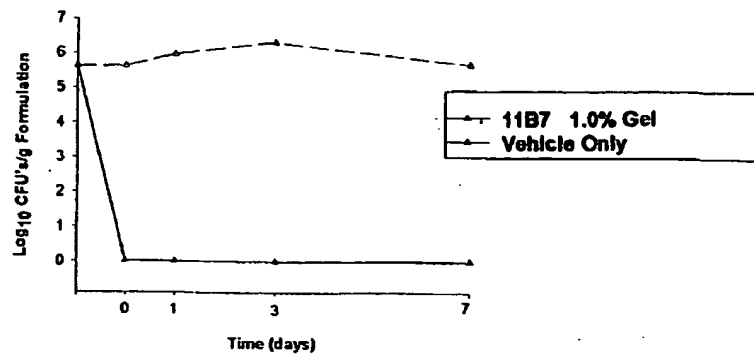
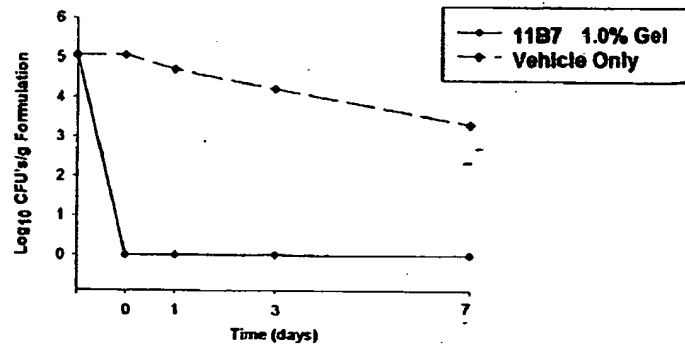
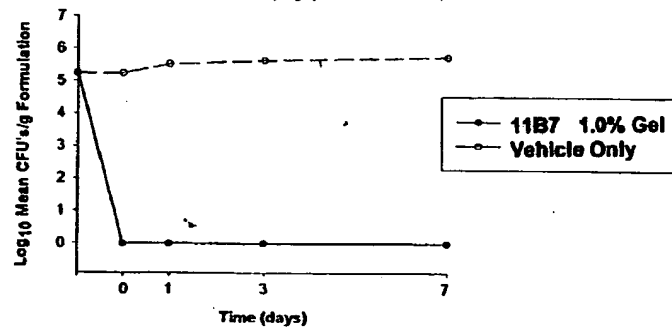
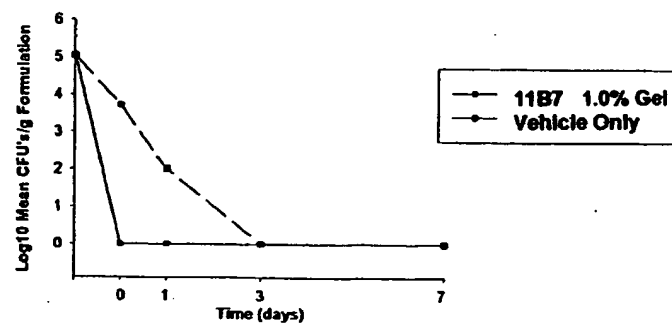
188. A method for treating or preventing infection at a target site, comprising applying to the target site a composition comprising a cationic peptide, a viscosity-increasing agent, and a solvent.

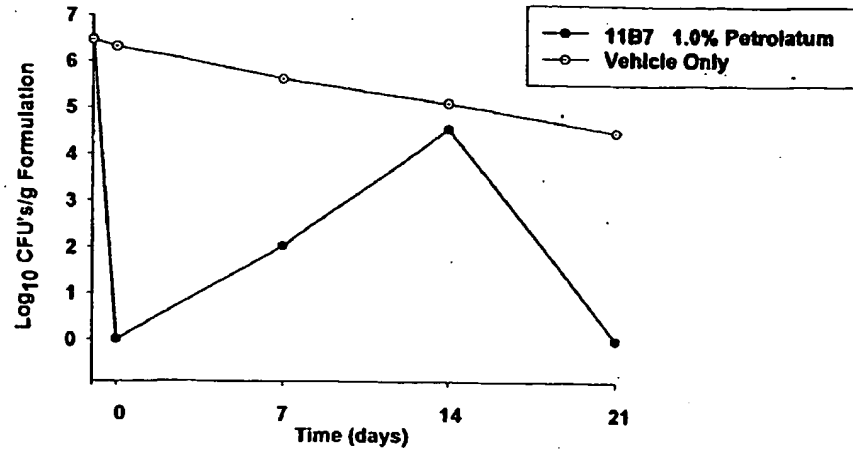
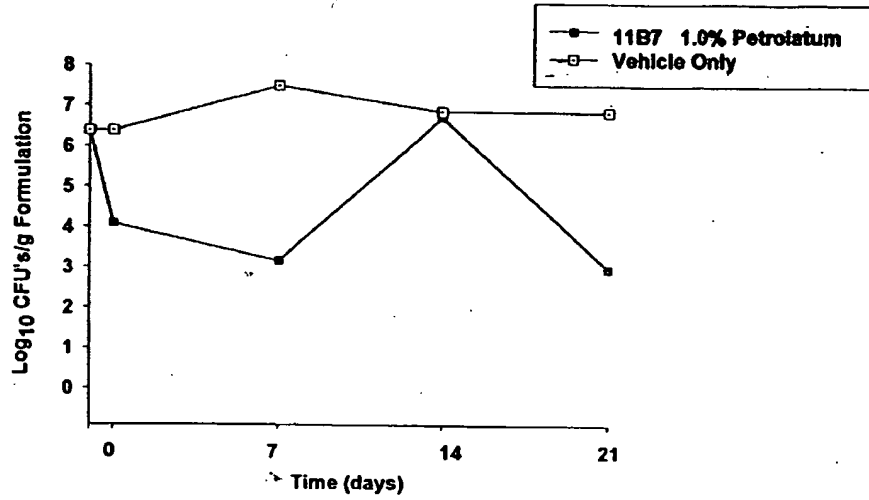
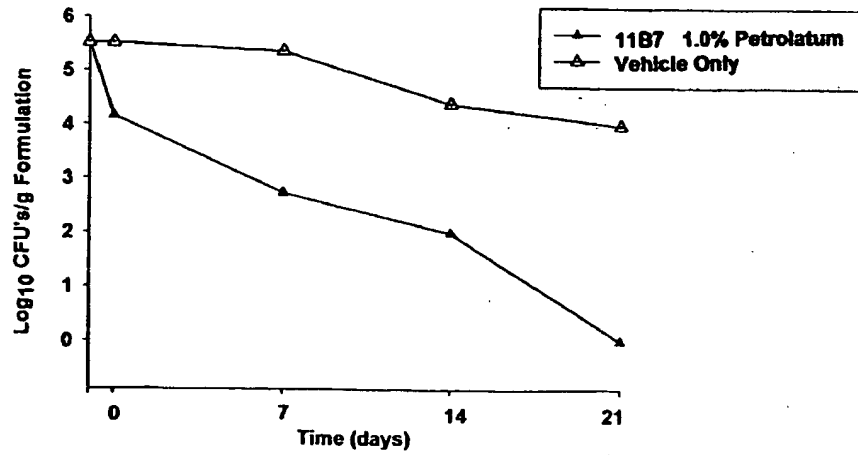
189. The method of claim 188 wherein the infection is caused by a prokaryotic organism, a eukaryotic organism, or a virus.

190. The method of claim 188 wherein the infection at a target site is associated with a medical device at the target site.

191. The method of claim 190, comprising applying the composition prior to or after inserting a medical device at the target.

192. The method according to claim 191 wherein the device comprises a catheter.

S. aureus SA016*S. epidermidis* SE010*C. albicans* CA002*P. aeruginosa* PA004**Figure 1**

E. coli*P. aeruginosa**S. aureus***Figure 2**